FATIGUE IN SKELETAL MUSCLE

BY

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The in situ dog gastrocnemius-plantaris muscle
preparation has been used to study fatigue. Skeletal
muscle fatigue (reduced force output for a given stimulus)
results from a thirty minute period of isometric
contractions at 2.5 to 20/sec. This fatigue is not a
result of failure of motor nerve propagation or transmitter
release. The ratio of oxygen uptake to developed tension
(total tension minus resting tension) is unaltered during
or following fatiguing contractions. The economy of force
production is unaltered by twin impulse stimulation,
relative ischemia or administration of moderate doses of
curare or succinylcholine.

When developed tension is reduced due to repetitive stimulation for thirty minutes at 2.5, 5 or 10/sec contractions, the time to peak tension and half relaxation times are unaltered. The peak rates of force development and of relaxation are reduced proportionally to the reduction in developed tension.

Following a forty minute period of recovery, the twitch developed tension remains greatly attenuated, but tetanic (200 msec of 100/sec stimulation) developed tension is virtually the same as it was before the stimulation period. Phosphorylcreatine is restored to resting levels within forty minutes of recovery. Also, at this time, blood flow and oxygen uptake have returned to pre-fatigue values and venous PO_2, PCO_2 and pH are at resting levels.

The fatigue observed in these experiments appears to be due to a reduction in the intensity of activation obtained with a single impulse. Energy sources are available and with maximal activation the contractile mechanism is capable of the same force output it had before the fatiguing contractions.

Further experiments were conducted to determine if intracellular acidosis could have been the cause of the reduced intensity of activation. A sixty to ninety minute period of hypoventilation with an air mixture high in O_2 (arterial PO_2 was maintained at 75-100 mm Hg) resulted in a reduction in arterial pH to 7.08. There was no reduction in twitch developed tension associated with this

acidosis. It is likely that intracellular pH fell as much during the respiratory acidosis as it did during fatiguing contractions at 10/sec. The fatigue observed during contractions at 10/sec could not be a result of intracellular acidosis.

It can be concluded from these experiments that twitch fatigue is not a result of energy deficiency, reduced capacity of the contractile elements, intra-cellular acidosis (induced by reduced ventilation for sixty to ninety minutes) or neuromuscular junction failure. By the process of elimination it appears that twitch fatigue results from a reduced activation of the myofilaments during a twitch contraction. This may be due to either a reduced sarcoplasmic Ca^{2+} concentration during contraction or a reduced response of the myofilaments at a given Ca^{2+} concentration.

INTRODUCTION

The word fatigue has been used in the past with
several various definitions. Some authors (1, 52) equate
fatigue with exhaustion. Others (47, 50) use the word
fatigue to represent an inability to maintain a particular
work output. More recently (23, 30) skeletal muscle
fatigue has been defined as a reduced capacity of the
muscle to develop tension. Edwards et al. (23) and Fitts
and Holloszy (30) have observed that a twitch contraction
can still be attenuated when the force generating capacity
of the muscle is fully recovered.

A single impulse does not maximally activate the
contractile apparatus (17) and therefore does not permit
the full contractile response of which the muscle is
capable. The capacity to develop tension must be
evaluated under conditions of maximal activation. This
can be accomplished with a caffein or K^+ induced
contracture or a maximal tetanic contraction.

If fatigue is defined as a reduced capacity of the
muscle to generate force, then what is it called if there
is an attenuated response to a single impulse? It appears
to be a separate phenomenon and probably occurs by a
separate mechanism (since a muscle recovers full capacity
to develop tension before twitch tension recovers to
pre-fatigue values). Edwards et al. point out (23) that

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this "twitch fatigue" may be associated with perception of increased effort necessary to maintain a given workload or force output. For the purposes of this dissertation, fatigue will be used as a generalization referring to a reduced response of the muscle to a given stimulation. Twitch fatigue will refer to an attenuated response to a single impulse.

Wilson and Stainsby (66) have reported that twitch developed tension of the in situ dog gastrocnemius-plantaris muscle remains attenuated for hours following a series of contractions at 10-14 per second. Fitts and Holloszy (30) have reported that tetanic developed tension recovers quickly following a period of repetitive stimulation. This may also be the case for the in situ dog gastrocnemius-plantaris muscle. If this is so, then the fatigue observed by Wilson and Stainsby is only twitch fatigue. This preparation, then, would provide a model for studying twitch fatigue independent of tetanic fatigue. Very little is known of the fatigability of the canine gastrocnemius-plantaris muscle or of the mechanisms responsible for that fatigue. The purpose of the present study was to provide further information regarding the fatiguing effects of repetitive stimulation on the gastrocnemius-plantaris muscle.

To facilitate the reader's understanding of skeletal muscle fatigue, a brief description of pertinent muscle physiology and current theories of fatigue precedes the sections describing the experiments which have been done.

The dog _in situ_ gastrocnemius-plantaris muscle group has been used throughout the series of studies reported herein. To avoid repetition, the general procedures and description of the preparation appear as a separate chapter before any of the studies. The specific procedures used in each study are described separately in a Methods section for that study.

In the first study, the relationship between oxygen uptake and developed tension has been determined for skeletal muscle before, during and after fatiguing contractions. These experiments were done to determine whether or not the energy used by a muscle relative to the amount of tension developed is altered by fatigue. There are reports that indicate a change in either direction can be expected (7, 21, 28).

Further studies were conducted to determine whether or not changes occur in the time-course of the twitch as a result of repetitive stimulation. Changes in the rate of force development and in the time-course of a twitch contraction have previously been interpreted as indications of changes in the duration and intensity of activation of the muscle (17, 36). These measurements may facilitate an understanding of the mechanism(s) responsible for the fatigue.

A third series of experiments has been conducted to study the effects of respiratory acidosis on the twitch contraction. Acidosis has been claimed to be one of

the major causes of fatigue (29). If this is the case, respiratory acidosis should reduce the developed tension of a twitch contraction.

In the final chapter of this dissertation, a brief discussion of the possible theories for the mechanism of fatigue observed in these experiments is presented. It is evident that further research will be necessary to permit evaluation of these theories with respect to fatigue of the canine gastrocnemius-plantaris muscle group. However, considerable evidence has accumulated as a result of my studies, which disputes several of the current theories of fatigue.

EVENTS LEADING TO
SKELETAL MUSCLE CONTRACTION

Muscular contraction is the result of a sequence of chemical and physical events beginning with activity in the central nervous system (CNS)(or sensory input to the CNS). Failure or impairment at any site in this process will result in a reduced contractile response of the muscle. Fatigue and twitch fatigue, then, are results of such failure. The identification of the site(s) of failure in fatigue would provide a better understanding of the mechanism(s) effecting the fatigue. Below, a brief discussion of the normal sequence of events leading to contraction is presented. CNS control of motor nerve activity is complex and will not be described. For simplicity, this discussion is based at the cellular level. This sequence of events is described in a number of text-books (45, 62) and is illustrated in Figure 1. Following the presentation of events leading to contraction, each step in the sequence is considered as a potential site for a mechanism of fatigue.

The sequence of events occurring at the nerve terminal may be susceptible to failure. The arrival of an action potential at the nerve terminal triggers the release of acetylcholine from the terminal bouton. Synaptic vesicles fuse to the terminal membrane and

release their contents into the synaptic cleft.
Acetylcholine diffuses the short distance across the
cleft (500 Å).

Binding of acetylcholine to specific receptors
causes a transient increase in permeability of the muscle
membrane to Na^+ and K^+. This results in depolarization
of the end plate. The resulting change in membrane
potential is called the end-plate potential. Destruction
of the acetylcholine is accomplished by acetylcholines-
terase which is located among the receptors on the post-
synaptic muscle membrane. Reconstitution of synaptic
vesicles is accomplished by reuptake of choline and
subsequent acetylation in the Golgi apparatus (enzyme:
choline-acetyl-transferase). Portions of the Golgi
complex, containing acetylcholine, are pinched off,
forming new synaptic vesicles.

A single action potential on a motor neuron usually
generates an end plate potential large enough to bring
the adjacent membrane area to threshold. Propagation of
an action potential over the membrane ensues. Transverse
tubules, located at regular intervals along the length of
the muscle fiber permit rapid communication with deep
portions of the muscle. Depolarization of transverse
tubules triggers release of calcium from the lateral sacs.
Lateral sacs are the terminal portions of the sarcoplasmic
reticulum lying adjacent to the transverse tubules. The
Ca^{2+} released from the lateral sacs raises the sarcoplasmic
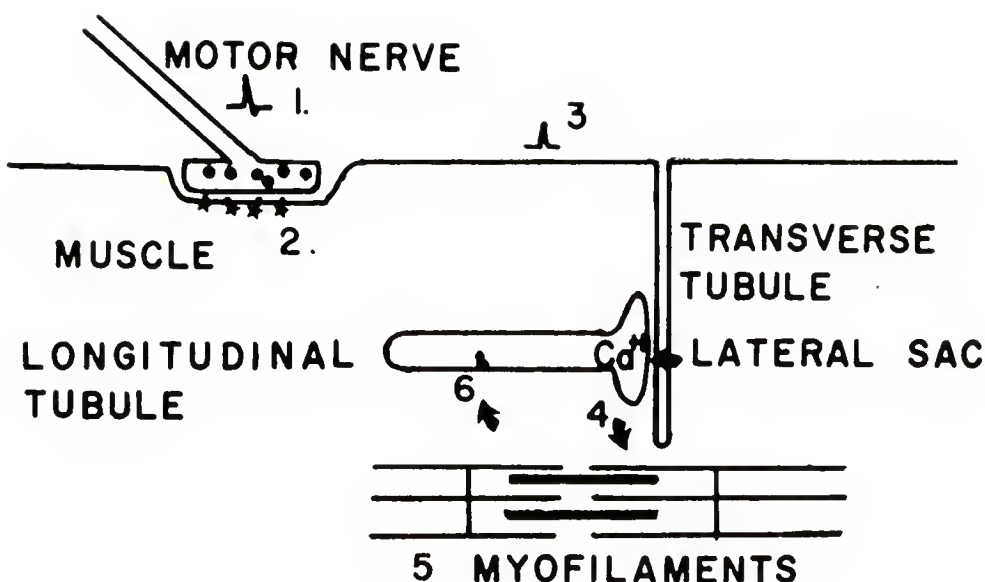
FIGURE 1.   The sequence of events occurring in
             excitation-contraction coupling are
             listed below. The numbers refer to
             numbered events shown in the diagram
             above.
             1.   An action potential travels along a
                  motor nerve.
             2.   Acetylcholine which has been released
                  from the nerve terminal binds to
                  receptors on the muscle membrane,
                  causing depolarization - an end-plate
                  potential.
             3.   When the end-plate potential reaches
                  a threshold value an action potential
                  is fired. This action potential is
                  propagated over the entire muscle
                  membrane, and causes depolarization
                  of the transverse tubules.
             4.   Depolarization of the transverse tubules
                  triggers release of Ca^{2+} from the lateral sacs.
             5. Ca^{2+} which has been released, binds to troponin
                  which is associated with the thin myofilaments.
                  Contraction results.
             6. Ca^{2+} is reaccumulated by an active transport
                  mechanism located in the longitudinal
                  tubules. Relaxation occurs.

free Ca^{2+} concentration. The subsequent binding of Ca^{2+} to troponin results in activation of the contractile proteins in the muscle. The amount of Ca^{2+} released in response to one action potential propagated over the muscle membrane is not sufficient to saturate the troponin molecules and therefore, incomplete activation occurs (17). For complete activation and therefore maximal force production, a period of nerve activity at a high frequency is necessary. Relaxation occurs as Ca^{2+} is sequestered (active transport) by the longitudinal sarcoplasmic reticulum. Following reuptake, calcium is translocated along the longitudinal reticulum to the lateral sacs, completing the Ca^{2+} cycle (67). The mechanism of this translocation is unclear.

POSSIBLE FATIGUE MECHANISMS AND
CURRENT THEORIES OF FATIGUE

Central Nervous System Fatigue

Events initiating muscular contraction originate
from sensory input or directly in the central nervous
system. Any study of fatigue during exercise of the
whole animal must consider the possibilities of central
inhibition resulting in reduced muscular performance.
There are conflicting reports concerning the potential
for a central component in muscular fatigue. For example,
Merton (44) found that maximal voluntary effort was not
different from the response of the muscle to maximal
tetanic stimulation of the motor nerve. He was studying
brief contractions of the adductor pollicis of humans.
Conversely, Asmussen and Mazin (1) have reported that
"diverting activity" (visual stimulation) permits greater
muscular performance than that which is accomplished when
the eyes are closed. Further experiments demonstrated
that immediate recovery from exhausting exercise (with
eyes closed) occurred if the eyes were subsequently
opened.

It is apparent from the work of Asmussen and Mazin
(1) that central effects can alter muscular performance.
It is important to keep in mind though, that under some

circumstances (i.e., brief maximal effort) fatigue appears
to be due entirely to peripheral mechanisms (44).

Neuromuscular Junction Failure

In the normal sequence of events preceding a muscular
contraction, an action potential is propagated over the
muscle membrane. The occurrence of a normal muscle action
potential is dependent on transmitter release and muscle
membrane properties. Repetitive stimulation may alter
these properties, and this could result in alterations in
the contractile response. Merton (44) found no change
in fatigue in the electromyogram resulting from maximal
stimulation despite an attenuation of force output.
Bergmans (3) studying human extensor digitorum brevis
observed no change in the surface electromyogram during
fatiguing contractions. Electromyography is not the
most sensitive technique for measuring the membrane
response, but any large alteration in muscle action
potential generation and propagation would probably
have been detected.

Using small muscle bundles, and measuring intracellular
potentials, Hanson (37) noted only minor changes in the
rat soleus muscle resting potential and action potential
following repetitive stimulation. The amplitude of the
action potential was reduced in fatigue, but was restored
within a few minutes of recovery. Grabowski (35) noted
a reduced amplitude of the muscle action potential of
fatigued frog muscle fibers. A reduced amplitude could

also be produced in a rested muscle by reducing extra-cellular Na^+ concentration. Under these conditions, twitch height is not altered. It would appear from the results of these experiments that following a period of fatiguing contractions, the amount of neurotransmitter released is sufficient to raise the end-plate potential to threshold, and the muscle membrane is capable of propagating a muscle action potential.

Attenuated Calcium Release

If a normal action potential is propagated over a muscle membrane, but less calcium is released from the lateral sacs, the contractile response will be attenuated. The reduced amount of Ca^{2+} released would result in a lower peak sarcoplasmic Ca^{2+} concentration and therefore a reduced activation. Direct measurement of Ca^{2+} release in a fatigued muscle has not been reported. Despite this, several authors have concluded that the mechanism responsible for the fatigue they observed was reduced Ca^{2+} release (15, 19). This conclusion is based on results from one of two techniques: either a) all other possibilities are eliminated or b) inference is obtained from analysis of changes in the time to peak tension and the peak rate of force development for a twitch. In the former, evaluation of the functional state of the neuromuscular junction and of the force generating capacity of the muscle has revealed that these processes are unaltered in the fatigued muscle. This leads one to

believe that the muscle has a reduced amount of Ca^{2+} released. In the latter, it is assumed that relaxation occurs simultaneously with Ca^{2+} reuptake (4). Under these circumstances, a reduction in contraction time would result from a reduction in duration of activation (more rapid reaccumulation or shorter duration of release). A reduction in peak rate of force development without a concommitant reduction in contraction time indicates reduced activation, and this is interpreted as a reduced amount of Ca^{2+} released. Brust has made observations similar to these (reduced rate of force development in fatigue with no change in contraction time) on mouse soleus muscles in vitro, (10) and concluded that fatigue was due to reduced Ca^{2+} release.

Similar observations would be expected if there was an increase in the Ca^{2+} concentration at which binding to troponin and subsequent contractile activity occurs. It has been observed by Fuchs et al. (32) that the affinity of troponin for Ca^{2+} can be altered by pH. This possibility must be considered when dealing with inferences from measurements of contraction time and rate of force development. Fitts and Holloszy (29) have presented data indicating that reduced pH may be associated with fatigue. They support the theory that reduced activation (and reduced rate of force development) is due to a reduced affinity of troponin for Ca^{2+}.

Another situation may occur in the muscle for which the rate of force development declines with developed tension while contraction time remains unchanged. A reduction in contractile capacity would give the same results. This possibility must be given consideration. Some authors have tested for, and found, changes in the contractile capacity of the muscle under study (30, 44). These are discussed below.

Reduced Capacity of the Contractile Apparatus

Fatigue may be the result of a reduced ability of the contractile proteins to generate tension. This could be a result of either: i) damage to myofilaments (i.e., misalignment or inactivation) or ii) restricted availability of energy. In either case, the effect would be a reduced force generation under conditions of maximal activation. This capacity to develop tension has been traditionally tested with either a K^+ contracture or a caffein contracture. Both of these procedures result in maximal activation (Ca^{2+} concentration high enough to saturate the contractile apparatus). Tetanic stimulation has also been used to evaluate the capacity of a muscle to generate tension.

Fitts and Holloszy (30) observed that tetanic force was reduced in the rat soleus muscle following a series of tetanic contractions. They noted that recovery of the force generating capacity occurred relatively quickly (within minutes). No insight into the mechanism

responsible for the fatigue observed by these authors is provided. Since recovery occurred quickly, it is obvious that permanent damage to the myofilaments was not a mechanism of the fatigue.

Spande and Schottelius (56) studied fatigue in the mouse soleus muscle in vitro. They found that the magnitude of the reduction in developed tension was inversely proportional to the phosphoryl-creatine (PC) concentration. PC serves as an immediate source of high energy phosphate (~P), for rephosphorylation of ADP and may also be involved in a transport capacity for ~P from mitochondria to myofilaments (40, 55). The experiments by Spande and Schottelius (56) involved contractions with periods of anoxia and/or glucose deprivation, and this must be kept in mind when comparing their results with those of other authors. Under these circumstances reduced energy availability appears to be related to the fatigue. Fitts and Holloszy (29) have measured PC changes during and following fatiguing contractions in rat muscle. They found no relationship between PC and the amount of fatigue or recovery from fatigue.

The final common mediator of energy availability is the level of ATP in the muscle. Edwards reported that ATP and PC concentrations were reduced in isolated mouse soleus muscles during prolonged tetani under anaerobic conditions. This was also the case when muscles were fatigued in the presence of cyanide and

iodoacetic acid. In the former case, lactate accumulated but in the latter case there was no accumulation of lactate. It was noted that prolongation of relaxation was associated with a reduction in ATP and PC levels. This provides an indirect method of evaluating energy availability in the muscle. Relaxation would be expected to be prolonged since it is dependent on reuptake of Ca^{2+}. Sequestering Ca^{2+} is an active transport process which requires ATP (13). Reduced levels of ATP may also slow the relaxation phase of individual cross-bridges. ATP is required to permit dissociation of the actin and myosin molecules (65). The extreme of this situation occurs when rigor bonds form in the absence of ATP.

It can be concluded from the above discussion that fatigue can be the result of any of several mechanisms. The possibility exists that multiple mechanisms function at once. For example, a reduced release of Ca^{2+} may be accompanied by a limitation of energy availability. This situation would complicate the elucidation of the mechanism(s) responsible for the fatigue.

The following chapters present the details of experiments conducted in an effort to gain an under-standing of fatigue in the gastrocnemius-plantaris muscle group of the dog.

GENERAL METHODS

Mongrel dogs of either sex weighing 9-18 kg were used in these studies. They were anesthetized with intravenous sodium pentobarbitol, 30 mg/kg, with additional 30 mg injections as needed. The animals were intubated and maintained on a respirator throughout the experiment. A Beckman LB-2 gas analyzer sampled gas from the endotracheal tube continuously. Ventilation was adjusted to maintain end-tidal CO_2 at 4.5 $\pm$ .25 %. Rectal temperature was monitored with a thermocouple, and kept between 37.5 and 38°C by appropriate adjustment of a heating pad placed under the thorax of the supine dog.

The left gastrocnemius-plantaris muscle was exposed via an incision along the medial aspect of the left hind limb. Muscles overlying the medial head of the gastrocnemius-plantaris muscle group were tied twice with butcher's cord and cut between the ties. These muscles are: sartorius, gracilis, semitendinosis and two heads of semimembranosis. All veins draining into the popliteal vein were ligated except those branches coming from the gastrocnemius-plantaris muscle (see Figure 2). Any veins draining the muscle but not entering the popliteal vein were ligated. These were

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FIGURE 2.   The in situ dog gastrocnemius-plantaris muscle
            preparation (58).
            G - gastrocnemius-plantaris muscle
            Gr- gracilis muscle
            S - sartorius muscle
            SM- two heads of semimembranosis muscle
            ST- semitendinosis muscle
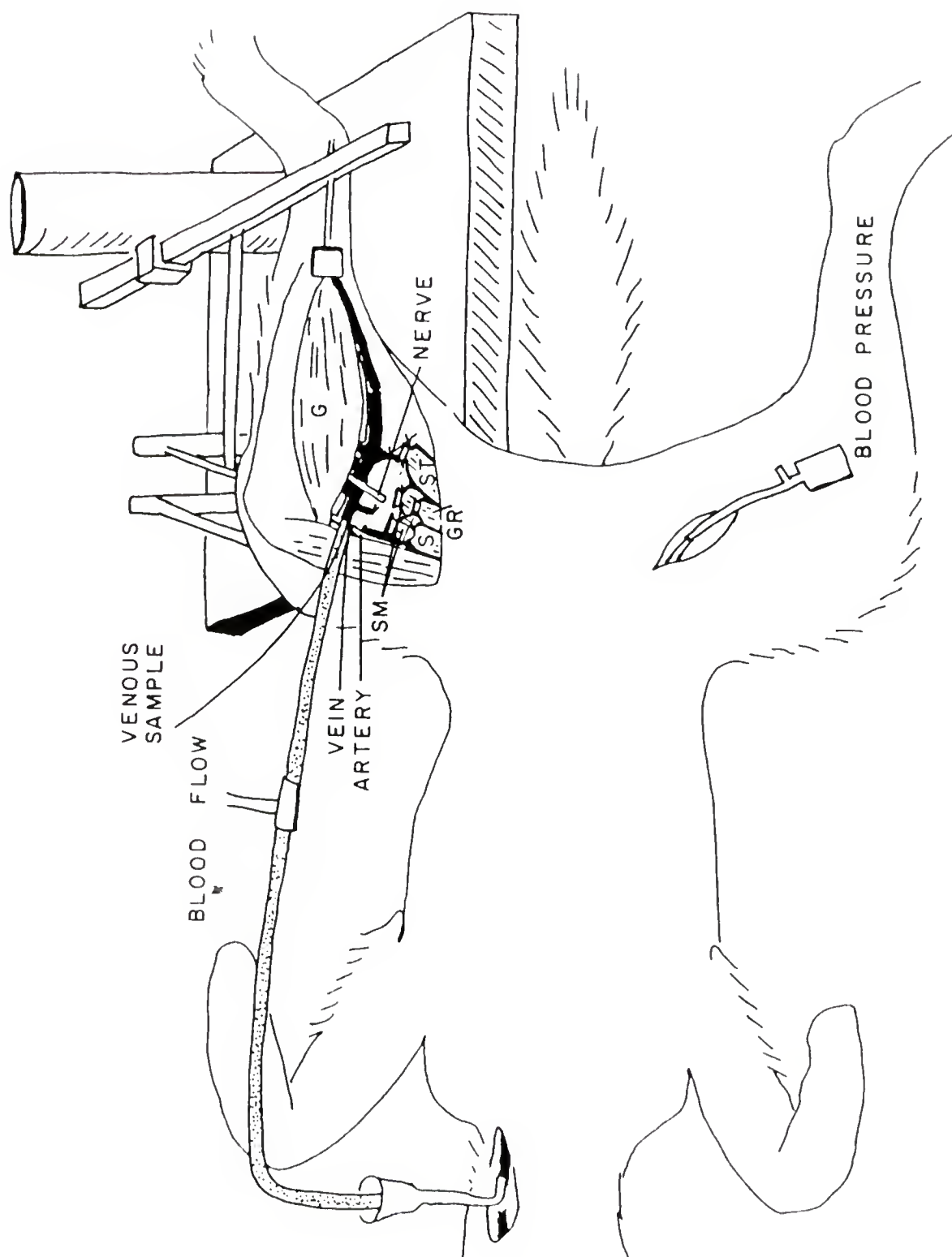
FIGURE 2

only minor vessels which occur along the anterior or
lateral surfaces of the muscle. The popliteal vein was
cannulated. A cannulating type electromagnetic flow
probe (Narco Biosystems) (3mm I.D.) was placed in the
outflow tubing. The venous effluent was returned to the
dog via another cannula in the external jugular vein.
Heparin, 2000 U/kg (12 mg/kg) was administered I.V.
initially and 1000 U/kg was given half way through the
experiment, prevented coagulation of blood in the tubing.
A thin cannula passed through the wall of the outflow
tubing and threaded within it to the muscle provided a
sampling port for venous blood. A thermocouple was placed
alongside this thin tube. The tip of the probe was
within 1 cm of the muscle. The blood temperature here
was assumed to be an average temperature of all parts of
the muscle. A heat lamp focused on the abdomen and hind
limbs was used to maintain muscle temperature near 37°C,
while the muscle was at rest. During contractions, the
lamp was turned off and the muscle temperature was
permitted to rise. The contralateral femoral artery was
cannulated and a Statham pressure transducer was connected
to the cannula. Output of the transducer was recorded
on a Grass polygraph model #5.

The Achilles tendon was severed close to the
calcaneous and securely fixed in an aluminum clamp.
The clamp was hooked to a slide bar which was fastened
to the cantilever beam of an isometric lever. Force

was measured with a displacement transducer detecting the displacement of the free end of the cantilever beam. The transducer output was linear for forces up to 20 kg. A displacement at the transducer of 0.1 mm gave a full scale deflection on the recorder. Output of the displacement transducer (tension) was amplified and recorded directly. The amplified tension signal was also differentiated with respect to time (Gould-Brush differentiator). The differentiated and direct signals were recorded on a Gould-Brush Model 2400 recorder. Blood flow and muscle temperature were also recorded continuously. The maximal rate of change of the amplified force signal never exceeded 80 v/sec. The differentiator was calibrated with ramp signals and was found to be linear through 120 v/sec.

The sciatic nerve was dissected free from surrounding tissue. All branches of the nerve not innervating the gastrocnemius-plantaris muscle were severed. The nerve trunk was double ligated about 4 cm proximal to the muscle and cut between the ties. A tubular stimulating electrode was placed on the distal stump of the nerve. The nerve was stimulated with a Grass Model SD9 stimulator with square pulses 0.2 msec in duration and of 2-4 volts. This voltage was double that necessary to produce a maximal contraction.

Contractions were isometric. The lever-arm of the myograph was bolted to a cast iron base which was

clamped to the table. Bone nails were placed in the tibia and femur (one each). These nails were firmly attached to the base of the myograph. A turnbuckle strut, placed between the lever-arm and one of the bone nails prevented flexing of the lever-arm. The muscle length was set 1-2 mm shorter than the length at which developed tension was greatest (optimal length). Optimal length was determined by measuring the developed tension (total tension minus resting tension) of contractions at (0.2/sec) at various lengths.

O_2 UPTAKE AND DEVELOPED TENSION

Introduction

Oxygen uptake ($\dot{V}O_2$) of muscle can increase more than 40 times resting levels during repetitive stimulation (57). At low frequencies of stimulation, $\dot{V}O_2$ is proportional to the isometric developed tension (ΔT) (total tension minus rest tension) (66). This relationship was observed for contractions following a period of fatiguing contractions at 10-14 per sec for 30 minutes (66). By stimulating the motor nerve with twin impulses, ΔT can be increased. It is not known whether the proportionality between $\dot{V}O_2$ and ΔT persists for twin impulses stimulation before or after fatiguing contractions. It is of interest to determine whether or not the muscle is capable of increasing its $\dot{V}O_2$ following fatigue, and if so, to see if ΔT is still proportional to $\dot{V}O_2$.

The purpose of this study is to investigate the effect of fatigue and twin impulse stimulation on the ratio between $\dot{V}O_2$ and isometric developed tension in the _in situ_ dog gastrocnemius muscle. Further experiments have also been conducted to determine the $\dot{V}O_2 : \Delta T$ relationship for muscle "fatigued" by curare infusion or ischemia during repetitive stimulation.

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Methods

The preparation described in the General Methods section was used in these experiments. Five series of experiments were completed to determine the relationship between muscle $\dot{V}O_2$ and ΔT.

Oxygen uptake by the muscle was calculated from the venous outflow and the arteriovenous blood oxygen content difference. Arterial samples were taken from the contralateral femoral artery. Venous samples were taken from the popliteal vein cannula via a thin catheter threaded through the wall of the venous outflow tubing to the end of the cannula close to the muscle group. The blood samples, 0.8 ml each, were collected in glass tuberculin syringes sealed with mercury-containing caps and kept in ice until analyzed for O_2 content with a Lex O_2 Con analyzer.

Series 1 and 2

Contractions began at the rate of 1/sec, and O_2 uptake and developed tension were measured after a steady level had been attained. Next, the muscle was fatigued by stimulating it at a rate of 10-20 impulses / sec for 30-40 minutes. This reduced the developed tension in a single twitch to about one-third to one-half of the pre-fatigue level. The muscle was allowed to recover for 30-40 minutes so that the resting $\dot{V}O_2$ approached the pre-fatigue level. Three pairs of blood samples were taken five minutes apart as the muscle continued to recover.

After this recovery period, the muscle was stimulated
at the same rate as before (1/sec) with twin impulses
(two impulses, 6.5 v in amplitude, 0.2 msec in duration
and separated by 10-20 msec), and O_2 uptake and developed
tension were measured. The time between the twin impulses
was set by adjusting the delay between impulses until a
smooth contraction was obtained. Post fatigue stimulation
with twin impulses returned the developed tension
approximately to the level of single impulse stimulation
pre-fatigue. In the second series of experiments, both
single and twin impulse contractions were done before
and after the fatiguing contractions.

In each type of contraction, the muscle was allowed
to contract for at least four minutes before arterial and
venous samples were taken to ensure that developed tension
and blood flow had reached a steady level. After an
additional two to three minutes of contractions, a
second pair of arterial and venous samples was collected.
The O_2 uptake rates calculated from the two pairs of
blood samples were averaged and the resting O_2 uptake
rate was subtracted to give the net O_2 uptake per minute.
This value was divided by the muscle weight and the
number of contractions per minute to give the O_2 uptake
in microliters of O_2 per gram of wet muscle per
contraction ($\mu l\ O_2 \cdot g^{-1} \cdot C^{-1}$). Developed tension was
expressed as grams of developed tension per gram of wet
muscle ($g \cdot g^{-1}$).

Series 3

Oxygen uptake and developed tension were measured during the fatigue process. In separate experiments, muscles were stimulated at rates of 3, 4, 5 and 6 impulses per second. After the first five minutes of contractions, blood samples were collected periodically as the muscle fatigued during contractions for two hours. The decrease in developed tension ranged from 34 to 45% over the two hour period. Sixty to 80% of this decrease occurred in the first 30 minutes. Although blood flow and developed tension were sometimes changing rapidly, there was almost no change in the arteriovenous blood oxygen content differences. This allowed application of the Fick equation for O_2 uptake calculation with confidence (64).

Series 4

Oxygen uptake and developed tension were measured in muscles during different levels of reduced blood flow produced by partially occluding the arterial inflow. The muscles were stimulated to contract at one twitch per second throughout these experiments.

Series 5

It is possible that a portion of the fatigue observed in the experiments of Series 1-3 might be due to presynaptic neural failure or neuromuscular junction failure, particularly in the first series of experiments in which the nerve-muscle preparation was stimulated at rates of 10-20 impulses / sec for 30-40 minutes. To

investigate this possibility, two experiments were done, in which neuromuscular transmission was completely blocked by repeated injections of either curare or succinylcholine. After the drug was given, the nerve was stimulated at the rate of 20 impulses / sec for 30 minutes. Muscle contraction did not occur during this 30 minute period because of the presence of the blocking drug. Developed tenion (at a stimulation rate of 1/sec) was measured before the drug was injected and after the effects of the drug had worn off. Therefore, any difference in developed tension before and after the period of high frequency stimulation with curare block would be due to either nerve or neuromuscular junction failure since the muscle did not contract during the 30 minutes of stimulation.

Neuromuscular fatigue was mimicked in a fresh muscle by infusing curare into the animal at different rates to block muscle contraction to varying degrees. O_2 uptake and developed tension were measured at the different levels of neuromuscular blockade. The stimulation rate was 1/sec.

Results

Resting O_2 uptake for the gastrocnemius-plantaris muscle averaged 7.7 µl $O_2 \cdot g^{-1} \cdot min^{-1}$. This is somewhat higher than average values previously reported (27, 57) but well within the usual range. Mean arterial blood pressure remained above 100 mmHg throughout all of the experiments.

In the first series of experiments, analysis of
variance for repeated measures (8) on the ratios
between O_2 uptake and developed tension observed before
and after fatigue revealed no significant difference
($p > .25$). Table I shows the O_2 uptake and developed
tension for each of the muscles both before and after
fatigue.

The results of the O_2 uptake and developed tension
measurements in series 2-5 are summarized in Table II
and illustrated in Figures 3 and 4. Table II shows the
linear regression equations relating O_2 uptake and
developed tension for each experiment. These equations
were calculated from data which included values from
the fatigued muscle as well as the fresh muscle. The
slopes of all but one (Experiment 8, $p = .09$) of the lines
are significantly different from zero ($p < .05$), despite
the small number of points used to determine each
regression equation. It is obvious from Figure 3 and
Table II that there was considerable variability between
animals. This has always been observed in this preparation
(27, 57, 66). However, despite differences in absolute
values between different animals, the same pattern of
response was observed in all cases. $\dot{V}O_2$ per contraction
and ΔT were directly related.

Results of four sample experiments from series 2-5
are shown in Figure 3. Figure 4 shows that all of the
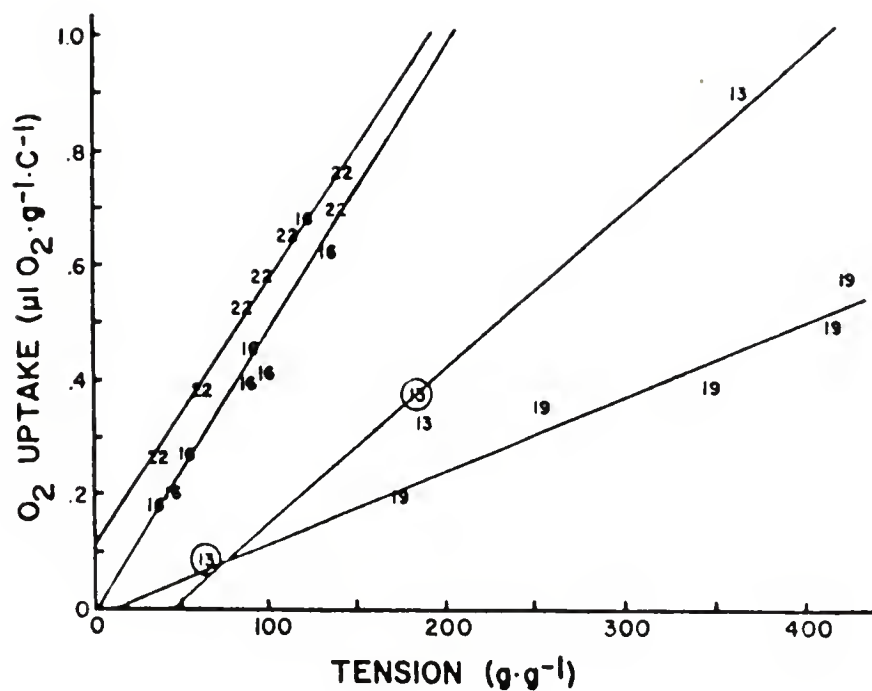
FIGURE 3. Results of four sample experiments.
Numbers refer to individual experiments.
Thirteen is from Series 2 (circled
numbers = post fatigue). Sixteen is
from Series 3. Nineteen is from
Series 4. Twenty-two is from Series
5.
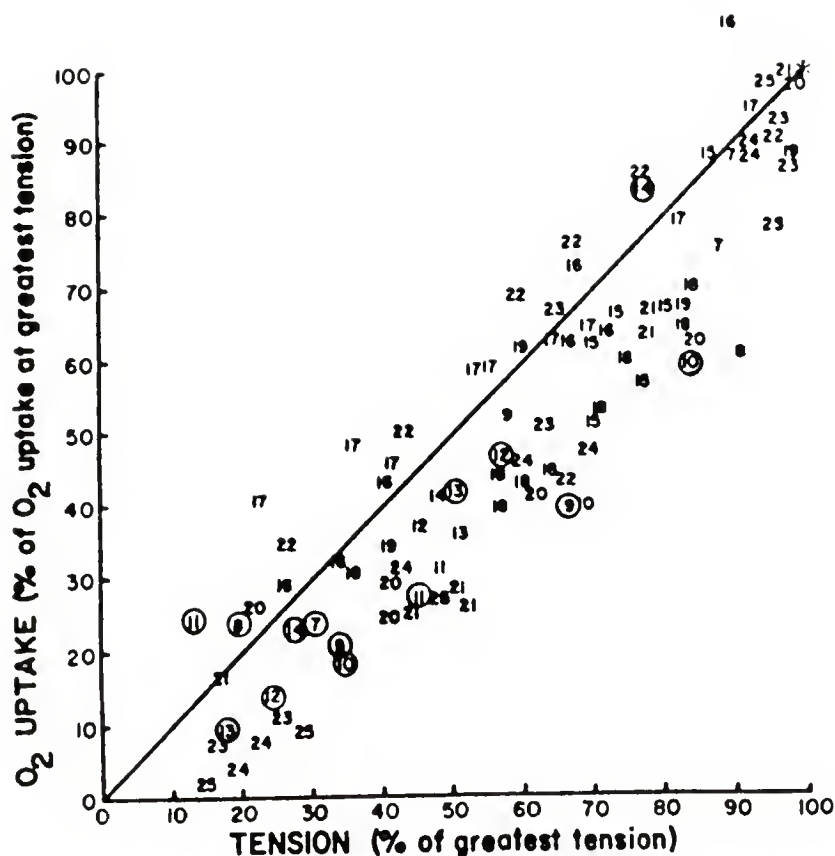
FIGURE 4. Data from Series 2-5 normalized to the same
scale. Developed tension in percent of the
greatest developed tension in each experiment.
O_2 uptake in percent of the O_2 uptake at the
greatest developed tension. Numbers refer to
individual experiments. Seven to fourteen are
Series 2 (circled numbers = post fatigue). Fifteen
to eighteen are Series 3. Nineteen to twenty-one
are Series 4. Twenty-two to twenty-five are
Series 5. The asterisk denotes (100%, 100%)
which is common to all of the experiments. The
line in this figure is the line of identity (X=Y).

TABLE I

O_2 UPTAKE AND DEVELOPED TENSION BEFORE AND AFTER FATIGUE IN SERIES 1

Experiment	Pre-Fatigue (Single Impulses)		Post-Fatigue (Twin Impulses)	
	Developed Tension ($g \cdot g^{-1}$)	O_2 Uptake ($\mu l\ O_2 \cdot g^{-1} \cdot c^{-1}$)	Developed Tension ($g \cdot g^{-1}$)	O_2 Uptake ($\mu l\ O_2 \cdot g^{-1} \cdot c^{-1}$)
1	148	.688	125	.361
2	196	.314	177	.356
3	254	.579	250	.446
4	134	.396	174	.435
5	207	.452	178	.258
6	219	.382	197	.476
Mean ± SEM	193 ± 18	.468 ± .057	184 ± 16	.389 ± .033

Units are as given for Figure 3

TABLE II

RELATIONSHIP BETWEEN O_2 UPTAKE AND DEVELOPED TENSION IN SERIES 2-5

*N equals the number of data points in each experiment

+ Units for O_2 uptake are microliters of O_2 per gram of wet muscle per contraction

Units for tension are grams per gram of wet muscle.

Series #	Expt. #	N*	Type of Fatigue	Regression Equation[+]	% Variance Explained
2	7	4	10/sec for 30 min	$\dot{V}O_2 = 3.3 \cdot 10^{-3}\ T - 9.3 \cdot 10^{-2}$	99.8
	8	4	10/sec for 30 min	$\dot{V}O_2 = 4.2 \cdot 10^{-3}\ T + 9.0 \cdot 10^{-2}$	83.4
	9	4	10/sec for 30 min	$\dot{V}O_2 = 3.6 \cdot 10^{-3}\ T - 2.6 \cdot 10^{-1}$	89.7
	10	4	10/sec for 30 min	$\dot{V}O_2 = 3.9 \cdot 10^{-3}\ T - 4.8 \cdot 10^{-1}$	89.5
	11	4	14/sec for 40 min	$\dot{V}O_2 = 2.8 \cdot 10^{-3}\ T - 2.0 \cdot 10^{-1}$	98.0
	12	4	20/sec for 30 min	$\dot{V}O_2 = 5.1 \cdot 10^{-3}\ T - 1.9 \cdot 10^{-1}$	99.6
	13	4	20/sec for 30 min	$\dot{V}O_2 = 2.7 \cdot 10^{-3}\ T - 1.2 \cdot 10^{-1}$	98.4
	14	4	15/sec for 30 min	$\dot{V}O_2 = 2.2 \cdot 10^{-3}\ T - 4.5 \cdot 10^{-2}$	98.6
3	15	7	Continuous at 3/sec	$\dot{V}O_2 = 4.0 \cdot 10^{-3}\ T - 2.8 \cdot 10^{-1}$	85.0
	16	8	Continuous at 4/sec	$\dot{V}O_2 = 5.0 \cdot 10^{-3}\ T - 8.2 \cdot 10^{-3}$	93.7
	17	10	Continuous at 5/sec	$\dot{V}O_2 = 3.4 \cdot 10^{-3}\ T + 8.1 \cdot 10^{-2}$	95.2
	18	10	Continuous at 6/sec	$\dot{V}O_2 = 3.0 \cdot 10^{-3}\ T - 9.1 \cdot 10^{-2}$	90.2
4	19	5	Ischemia	$\dot{V}O_2 = 1.3 \cdot 10^{-3}\ T - 1.3 \cdot 10^{-2}$	91.6
	20	6	Ischemia	$\dot{V}O_2 = 4.6 \cdot 10^{-3}\ T - 7.6 \cdot 10^{-2}$	88.9
	21	8	Ischemia	$\dot{V}O_2 = 5.1 \cdot 10^{-3}\ T - 2.2 \cdot 10^{-1}$	91.6
5	22	7	Partial Curare Block	$\dot{V}O_2 = 4.6 \cdot 10^{-3}\ T + 1.1 \cdot 10^{-1}$	97.2
	23	8	Partial Curare Block	$\dot{V}O_2 = 1.5 \cdot 10^{-3}\ T - 7.2 \cdot 10^{-2}$	97.7
	24	8	Partial Curare Block	$\dot{V}O_2 = 5.5 \cdot 10^{-3}\ T - 1.9 \cdot 10^{-1}$	97.7
	25	7	Partial Curare Block	$\dot{V}O_2 = 5.2 \cdot 10^{-3}\ T - 2.6 \cdot 10^{-1}$	95.9

data follow the same pattern when normalized to the same scale. In this figure, developed tension is plotted as the percent of the highest tension developed in each individual experiment, and O_2 uptake is plotted as the percent of the O_2 uptake at the highest developed tension. Most importantly, Figures 3 and 4, and Tables I and II show that the relationship between O_2 uptake and developed tension was unchanged by the various treatments.

In two experiments, muscle contraction was completely blocked by repeated injections of curare or succinylcholine while the nerve was stimulated 20 times per second for 30 minutes. Injection of the blocker was discontinued after the stimulation period and the effects of the blocker were mostly dissipated within 10 minutes. Developed tension was still at least 90% of the control value. The observed reduction in contraction strength may have been due to incomplete recovery from the neuromuscular block. This 10% reduction in developed tension can be compared with the 50-70% reduction observed in the other experiments in which muscle contraction was not blocked. It appears that most if not all of the reduced contractile response was due to alterations beyond the neuromuscular junction.

As pointed out in the Methods, the fatigued muscles in the first and second series of experiments were allowed to recover for 30-50 minutes. After this time, the resting O_2 uptake approached the pre-fatigue level.

However, developed tension recovered very little during this time and was still only one-third to one-half of the pre-fatigue value.

Discussion

Isometric developed tension at constant muscle length was varied in this study by four methods: 1) twin impulses stimulation, 2) fatigue produced by 30 minutes of contractions at 20/sec, 3) ischemia caused by partial occlusion of arterial inflow to the muscle, and 4) partial block of neuromuscular transmission with curare. Figures 3 and 4, and Tables I and II show that none of these treatments changed the relationship between O_2 uptake and developed tension. Stimulating the fatigued muscle with twin impulses restored developed tension to pre-fatigue values. Fatigue did not increase the O_2 requirement per unit of force developed, even when the tension developed by the fatigued muscle was returned to the pre-fatigue level by twin impulses stimulation. The fatigued gastrocnemius-plantaris muscle is therefore capable of increased developed tension and increased $\dot{V}O_2$. In addition, the O_2 requirement per unit of force developed was not altered during the development of fatigue.

The dog gastrocnemius-plantaris muscle group has certain advantages in studies of fatigue. Based on histochemical staining properties, the dog gastrocnemius contains only two motor unit types (43). These two

correspond to types FR and S (SR), (fast, fatigue
resistant and slow, fatigue resistant respectively),
described by Burke and colleagues (11) for hindlimb
muscles of the cat. Even though the dog gastrocnemius-
plantaris muscle group contains both FR and S units,
and the cat soleus muscle contains only type S units,
homogenates of cat soleus muscle have less than one-
third of the succinate oxidase activity of homogenates
of the dog gastrocnemius-plantaris muscle group (43).
From this, one might expect all of the dog gastrocnemius-
plantaris muscle units to be more resistant to fatigue
than any of the units of cat soleus muscles. However,
Burke and colleagues (11) have warned against extra-
polation of histochemical and biochemical properties
to physiological properties.

There are several possible causes of the fatigue
observed in these experiments. In Series 1-3, fatigue
could have resulted from a failure in excitation-
contraction coupling, substrate depletion, accumulation
of metabolites, or a combination of these factors.
Since there are both FR and S fiber types in the
gastrocnemius, the fatigue might have been predominantly
in one of the fiber types.

It seems unlikely that neuromuscular junction failure
was a significant component of the fatigue observed in
Series 1-3. Testing for neuromuscular transmission
failure by direct stimulation of the dog gastronemius-
plantaris muscle group is not easy since its large size

makes constant field stimulation difficult. However, several studies on other mammalian muscles (3, 41, 52) have indicated that the possibility of neuromuscular transmission failure at stimulation rates of less than 10/sec is minimal. In two experiments, nerve stimulation at 20 impulses / sec for 30 minutes when muscle contraction was blocked by curare or succinylcholine caused less than a 10% decrease in developed tension. Decreases in developed tension of 50-70% occurred under the same stimulation conditions when muscle contraction was not blocked. These findings indicate that presynaptic failure of impulse propagation and inadequate release of acetylcholine probably did not cause the fatigue observed in our experiments. Desensitization of the endplate is not ruled out by these results. However, neuromuscular depression is presently believed to result from a reduced number of released transmitter quanta and a reduction of quantal size (42, 48).

In Series 4, the cause of fatigue might have been muscular, neuromuscular, or a combination of the two since ischemia can affect both the muscle and the neuromuscular junction (16, 49). In Series 5, developed tension was decreased by partial curare block which presumably simulates neuromuscular junction failure.

These experiments do not allow identification of the specific cause of fatigue. However, our results do indicate that the oxygen uptake per unit of isometric

force production is unchanged by either muscle fatigue or neuromuscular fatigue. This suggests that fatigue, whether muscular, ischemic, neuromuscular, or a combination of these three, does not cause any change in the efficiency of energy transduction from ATP to external tension development by the muscle, without concommitant changes in the opposite direction for energy transduction from foodstuffs to ATP. This is not likely the case.

These results differ from those of Bronk (6), Feng (28), Edwards and Hill (20) and Edwards, Hill and Jones (21) in that they found that the energy expenditure per unit of force production (or of tension-time) decreased during fatigue. Unlike our experiments, however, these earlier studies used stimulus parameters which caused partially to completely fused tetanic contractions of relatively long duration. The present study is of twitch or very brief tetanic contractions, for which no plateau in developed tension occurs (see Figure 5). There is little if any tension maintenance involved.

The data presented in this study along with those of Wilson and Stainsby (66) demonstrate a constant coupling between O_2 uptake and developed tension in isometric twitch contractions. In these two studies, developed tension has been changed by stimulation frequency, potassium ion infusions, twin impulse

FIGURE 5. Tracing of a recording of tension and
differential of tension for single impulse
and twin impulse contractions before and
after fatigue. Contractions are superimposed
for ease of comparison. There is no
maintained plateau in this type of
contraction.

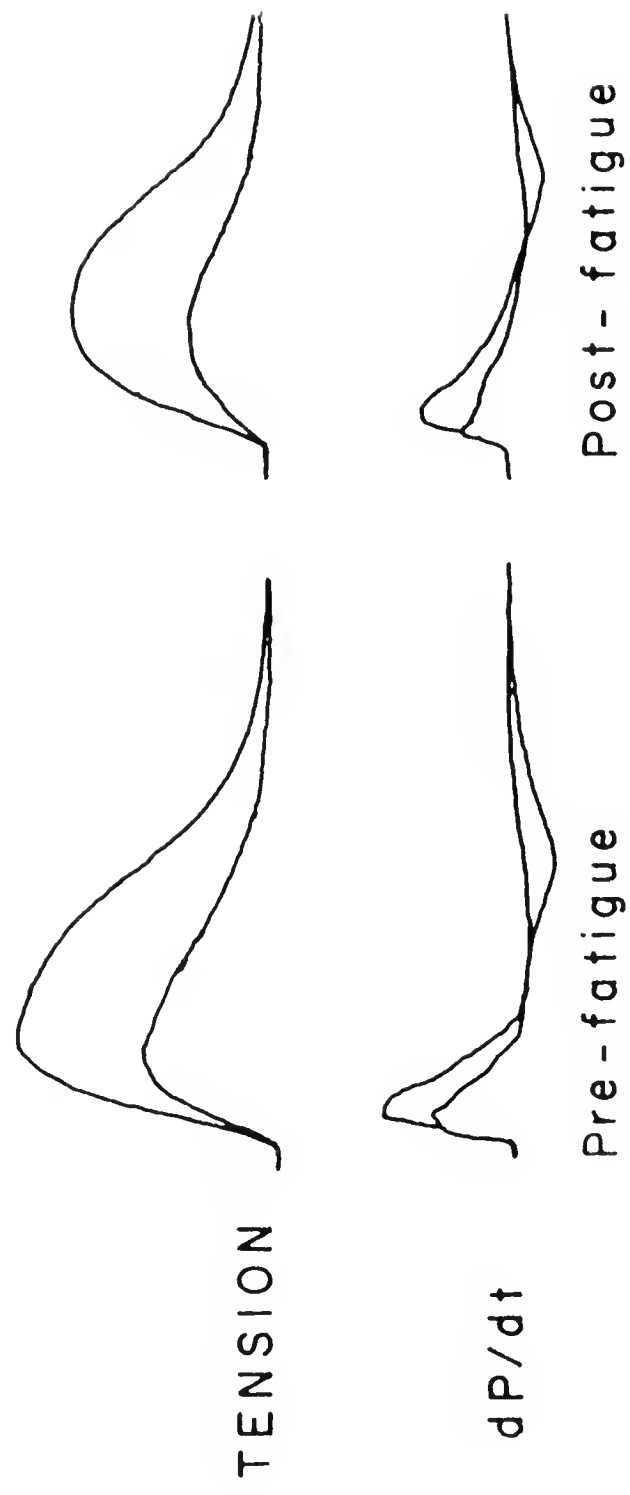TWITCH and TWIN CONTRACTION

FIGURE 5

stimulation, normal muscle fatigue, ischemic fatigue and partial neuromuscular transmission block with curare. During all of these treatments, the relationship between O_2 uptake and developed tension has been unaltered.

The data also show that although resting metabolic rate following fatigue approaches pre-fatigue levels after 30 minutes, developed tension is still quite low. Phosphorylcreatine and ATP levels should be fully recovered following 30 minutes of rest (38, 51). Edwards and coworkers (23) have also identified a long lasting element of fatigue in humans that is not due to depletion of high-energy phosphates. Further study is warranted to determine whether or not there really is a causative relationship between phosphorylcreatine depletion and fatigue, as suggested by Spande and Schottelius (56).

EVALUATION OF CHANGES IN THE TWITCH CONTRACTION
ASSOCIATED WITH FATIGUE

Introduction

Skeletal muscle has an attenuated response to a single impulse following a prolonged period of twitch contractions due to repetitive single impulse stimulation (34, 66). This response is not necessarily indicative of a reduced capacity of the muscle to develop tension (23, 30). It is, however, a type of muscular fatigue and warrants further investigation concerned with determination of mechanisms responsible for this "twitch fatigue."

Wilson and Stainsby (66) reported that twitch fatigue occurs in the gastrocnemius-plantaris muscle of the dog following 30-40 minutes of isometric contractions (10-14/sec). They monitored recovery with periods of low frequency stimulation over the course of 3-4 hours. An attenuation of developed tension was still present following this recovery period. Little is known of the mechanism responsible for this fatigue.

The purpose of the present investigation was to study alterations in twitch contractions caused by repetitive stimulation at three frequencies; 2.5, 5 and 10/sec. A twitch contraction can be characterized

41

by measurements of the magnitude and time-course of
tension development seen in the isometric myogram (3, 9).
These measurements are: developed tension (ΔT),
contraction time (Ct), half relaxation time (Rt 1/2),
peak rate of force development (dP/dt), and peak rate
of relaxation (-dP/dt) (see Figure 6). Sandow and Brust
(54) have named the changes in these measurements that
occur with repetitive stimulation the "fatigue patterns."
Fatigue patterns have been determined for single muscle
cells and whole mammalian and amphibian skeletal muscles
in vitro (9, 10, 35). Measurements on in situ muscle
where direct determination of muscle force during
repetitive stimulation can be made while the muscle
maintains a normal circulation have not yet been
reported.

Methods

Twenty mongrel dogs of either sex weighing 9-18 kg
were used in this study. The gastrocnemius-plantaris
muscle group was prepared as described in the General
Methods chapter.

Fatigue as a result of 30 minutes of stimulation at
three frequencies 2.5, 5 or 10/sec was studied. Five
animals were used at each frequency. To study the fatigue
patterns of muscle, it is necessary to obtain fast traces
of contractions, before, during and after the fatiguing
contractions. To evaluate twitch contractions before
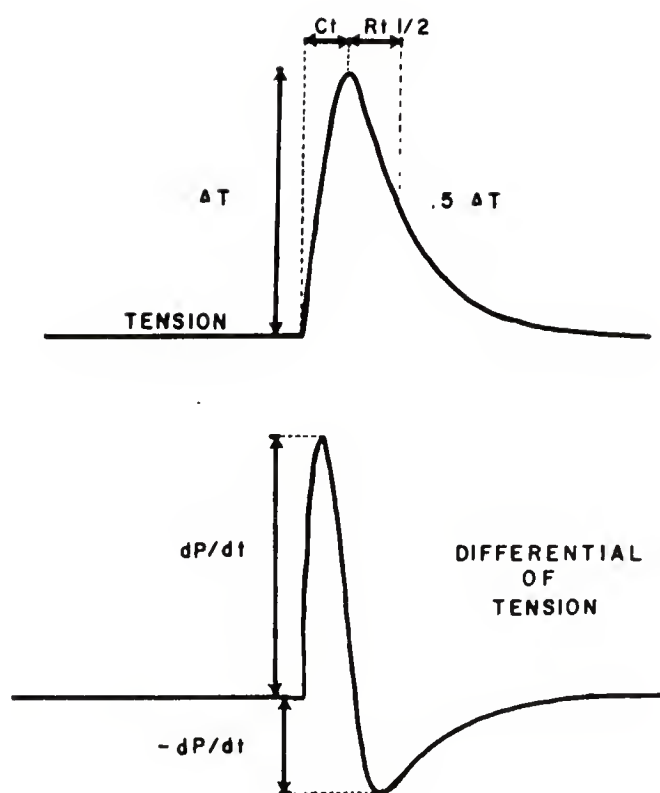fatigue, the muscles were stimulated at either 1/sec or

Figure 6. Tracings of: Tension and dP/dt are presented to demonstrate the manner by which the measurements were made. See text for verbal description of these terms.

2.5/sec for 2 minutes. After a fast trace (100 or 200 mm/sec paper speed) was obtained, contraction frequency was either left at 2.5/sec or increased from 1/sec to 5 or 10/sec. Relaxation was not complete between contractions when stimulation was 5/sec or 10/sec. To facilitate measurement of the characteristics of a twitch, the frequency of stimulation was reduced briefly, while fast traces were obtained, then the fatiguing frequency was restored (see Figure 7). During contractions at 2.5/sec complete relaxation occurs between contractions, so fast traces were obtained without altering the frequency of stimulation. Besides the contractions at 2 minutes, fast traces were obtained after 10 and 30 minutes of fatiguing contractions and after 10 and 40 minutes of recovery (see Figure 7). To get fast traces during the recovery period which followed contractions at 5/sec or 10/sec, the stimulator was turned on briefly at 1/sec. Following the 30 minute period of contractions at 2.5/sec, contractions were continued at a frequency of 0.2/sec. Fast traces were obtained without altering the frequency of stimulation. It has been reported that contractions at this low frequency do not alter the recovery process (66). In one experiment, a tetanic contraction (200 msec duration, 100 impulses / sec) was obtained, before and after the 10/sec fatiguing contractions. This was done to permit evaluation of the contractile capacity of the muscle. All fast traces were evaluated

for the characteristics of a twitch. These characteristics are illustrated in Figure 6.

Arterial and venous blood samples (0.6 ml) were obtained at regular intervals throughout the experiment. Samples were drawn into glass tuberculin syringes, sealed with mercury-containing caps, and placed in ice until they were analyzed. These samples were analyzed for pH, PCO_2 and PO_2 at 37°C with a radiometer (Copenhagen) blood gas machine. These measurements permit evaluation of viability of the animal and provide descriptive data concerning metabolic status of the muscle.

At the end of each experiment, the fatigued muscle was excised, trimmed of visible fat and connective tissue, blotted and weighed. The force transducer was calibrated after each experiment by hanging pre-weighed lead weights on the lever.

In a few additional experiments, twitch contractions were evaluated when ΔT was reduced by: ischemia, dantrolene sodium or reduced stimulation voltage. Comparison of these contractions with those obtained during and/or following fatiguing contractions may provide some insight into the mechanism of fatigue. To study the effects of ischemia, the femoral artery was occluded while contractions continued at 1/sec. Fatigue would not occur at this frequency with an intact blood flow, but does occur with ischemia. The occlusion was removed after ΔT fell to about 50% of the pre-occlusion value (10-20 minutes)
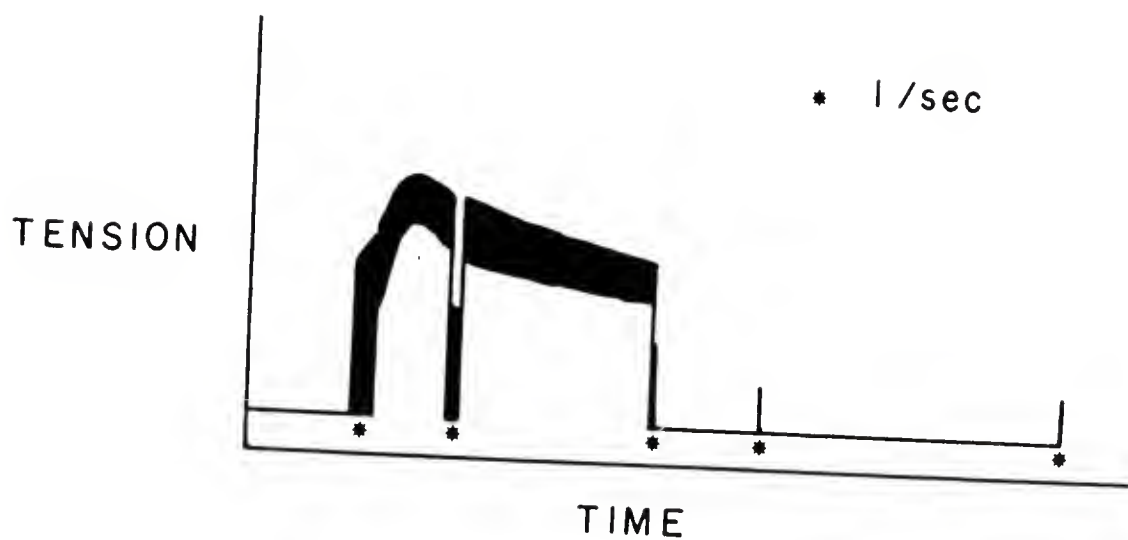
FIGURE 7.   Tension developed versus time.   Contractions
in this case were 10/sec except as indicated.
Fast traces (not illustrated) were obtained
during the 1/sec stimulation.

and recovery was observed. Dantrolene sodium, dissolved in propylene glycol (25 mg/ml) was injected I.V. during contractions at 0.2/sec. Dantrolene impairs release of Ca^{2+} from the lateral sacs (24). This is accomplished without changes in the action potential and is apparently a direct effect on the lateral sacs. Sufficient drug was given to reduce ΔT at least 50% (2-5 mg/kg). To study the effects of reducing the number of motor units contracting, the stimulation voltage was reduced while the muscle contracted 0.2/sec. This results in excitation of fewer motor neurons and their motor units. Consequently less tension is developed. Comparing the twitch characteristics of a normal versus a fatigued muscle may provide information leading to an understanding of the mechanism(s) of fatigue.

Also, in a few experiments, samples of muscles were obtained immediately following the 30 minute stimulation period, and/or after 40 minutes of recovery. Samples were frozen in situ with metal clamps pre-cooled in liquid nitrogen. Small samples (30-80 mg) were then homogenized (Vertis homogenizer) in perchloric acid (.8% in 40% ethanol) and analyzed for phosphorylcreatine by the method of Ennor and Stocken (25) (see Appendix).

Statistical analysis was by the two way analysis of variance for repeated measures. Differences between means were determined by Duncan's multiple range test (2).

Results

Blood samples were obtained before the contractions began and at $t = 10, 30, 40$ and 70 minutes. Arterial PO_2 was 87 ± 2.3 mm Hg (mean $\pm$ SEM) before contractions and did not change significantly throughout the experiments (see Figure 8). Before contractions began, PvO_2 was 50.2 ± 2.0 mm Hg. During the contraction period PvO_2 was lower, but none of the blood samples measured had a PO_2 less than 12 mm Hg. Except for the experiments where fatigue was caused by $2.5/sec$ contractions, PvO_2 was back to pre-fatigue values early in the recovery period. Contractions were continued, $0.2/sec$, during the recovery period of these ($2.5/sec$) experiments; therefore, it might be expected that PvO_2 would not be at rest levels.

Arterial PCO_2 began at 31.6 ± 0.8 mm Hg and fell slowly during the experiments. The decrease in $PaCO_2$ was statistically significant but probably is of minimal physiological significance. Venous PCO_2 was high during the contraction period when PvO_2 was low, and returned to pre-contraction levels early in the recovery period.

Arterial pH was 7.40 ± 0.01 before contractions began, and did not change significantly throughout the experiments. Venous pH decreased from 7.37 at $t = 0$ minutes to 7.32 (for $2.5/sec$) or 7.28 (for 5 or $10/sec$) at $t = 10$ minutes. By 10 minutes of recovery, venous pH had returned to pre-fatigue values (see Figure 8).

FIGURE 8. Blood flow and the blood gas measurements
versus time. The horizontal bar
indicates where fatiguing contractions
occurred. When means at a given time
(for different frequencies) were not
significantly different, means were
combined. Numbers refer to the
fatiguing frequency. Asterisks
indicate where measurements are
significantly different from the
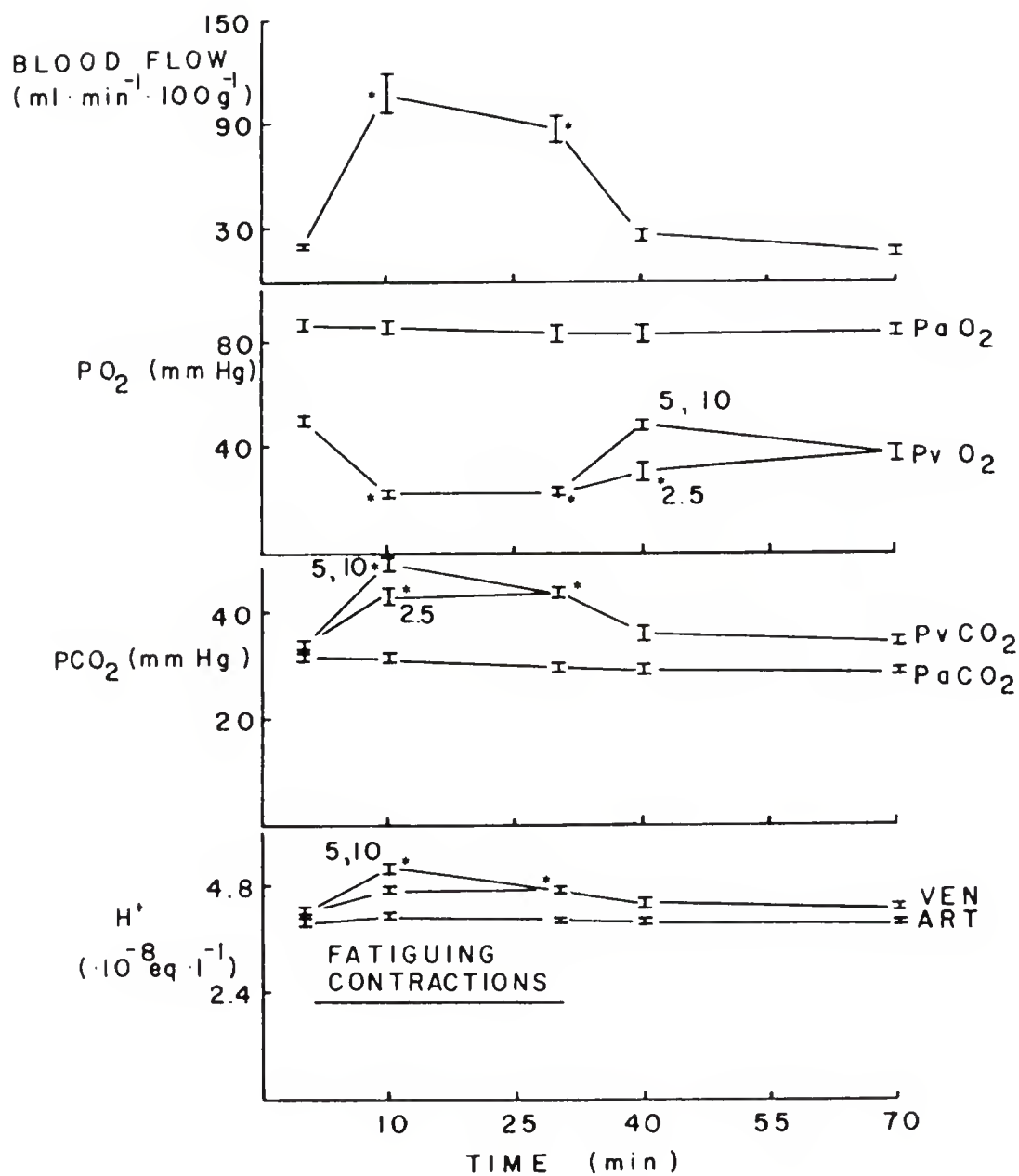original value (time = 0 minutes).
Vertical bars are $\pm$ SEM.

FIGURE 8

Although blood flow was measured continuously, only those measurements corresponding to times when blood samples were obtained are presented (see Figure 8). Blood flow was higher during the contractions, but was back to pre-fatigue values by 10 minutes of recovery. There were no significant differences between frequencies for blood flow response.

The first 2 minutes of contractions were at 1/sec or 2.5/sec. There were no significant differences for ΔT between these frequencies at t = 2 minutes. Mean ΔT for all experiments was 2.3 $\pm$ 15 g/g (wet wt) at this time. The muscles weighed 48.5 $\pm$ 3.3 g (wet wt). Developed tension fell more rapidly during 10/sec contractions than during 2.5/sec or 5/sec contractions (see Figure 9). By 30 minutes all frequencies of stimulation resulted in significant reductions in ΔT. There was no significant recovery of ΔT during the 40 minutes following the fatiguing contractions.

Contraction time decreased during the fatiguing contractions at 10/sec, but not during the contractions at 2.5 or 5/sec. There was no significant difference for Ct between recovery and pre-fatigue measurements at any fatiguing frequency (see Figure 9).

Half relaxation time did not change during the contractions or during the recovery except for recovery of 2.5/sec fatigue. The Rt 1/2 was longer for contractions at 0.2/sec than for 1/sec. If contractions during recovery

FIGURE 9.   Developed tension, contraction time
            and half relaxation time versus time.
            The horizontal bar indicates when
            fatiguing contractions occurred.
            Where there was no significant
            difference between means, all
            frequencies are combined. Numbers
            refer to the fatiguing frequency.
            Asterisks indicate where measure-
            ments are significantly different
            from the original value (at time =
            2 minutes). Vertical bars are $\pm$ SEM.

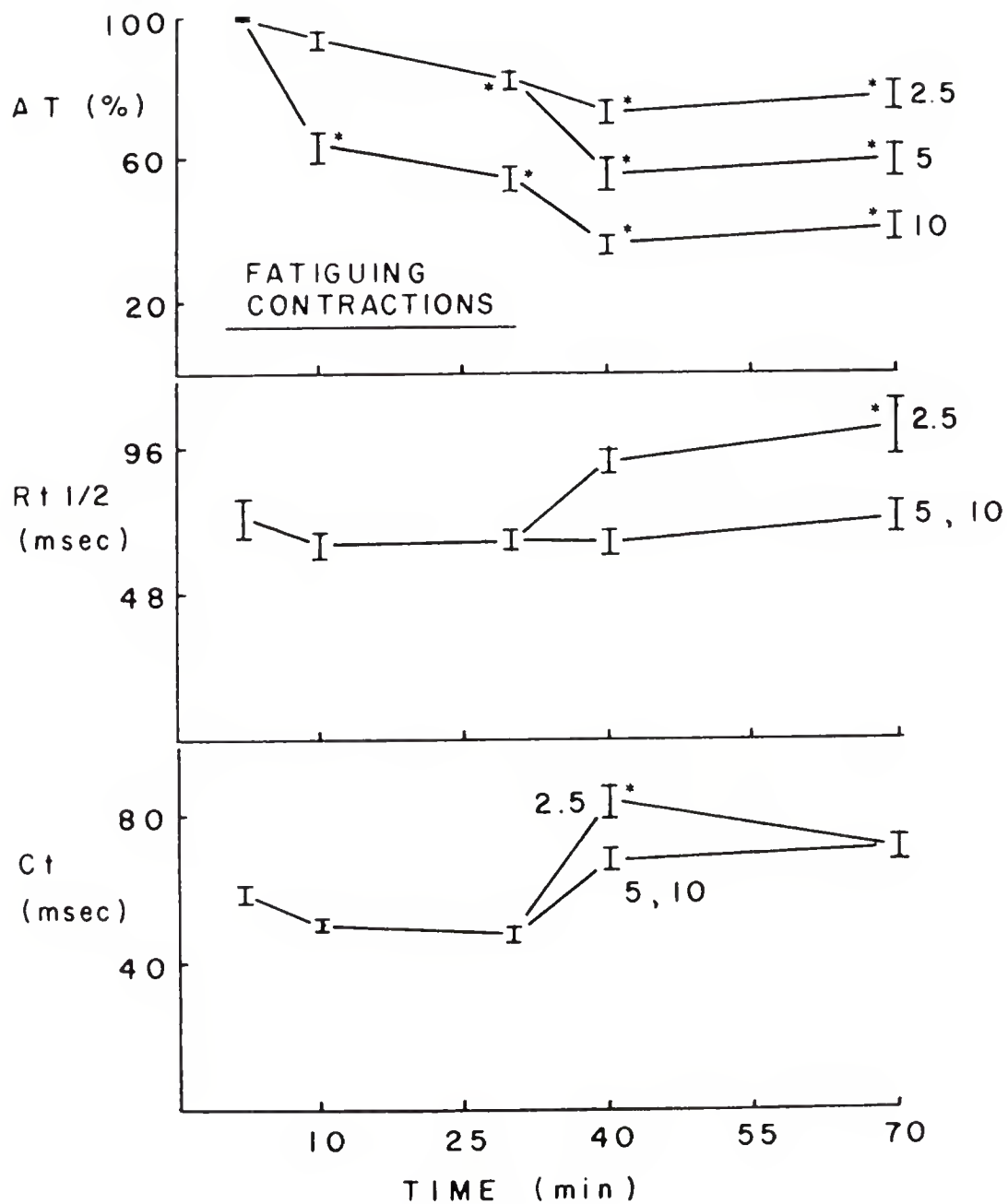FIGURE 9

for this frequency of fatiguing contractions had been 1/sec (at t = 40 and 70 minutes only) then no difference from pre-fatigue contractions would be expected.

Figure 10 illustrates the changes in dP/dt and -dP/dt seen in these experiments. The changes seen for dP/dt closely parallel those observed for ΔT. A positive and significant correlation exists between dP/dt and ΔT ($r^2 = 0.93$) and between -dP/dt and ΔT ($r^2 = 0.82$).

Muscle temperature rose during the fatiguing contractions. The increase in temperature was only 1-2°C. A similar or smaller rise was seen during the 5/sec and 2.5/sec contractions. Muscle temperature fell slowly to 37°C during recovery, but was not permitted to go below 37°C.

Muscle samples obtained during a few of the experiments were analyzed for phosphorylcreatine (PC). Analysis revealed that PC is low at t = 30 minutes (during contractions), but is back to resting levels by t = 70 minutes (see Table III). The values of PC given in the table are left:right ratios. PC was determined relative to total creatine in the muscle sample. Harris (38) has shown that total muscle creatine content does not change during exercise and therefore can be used as an index of muscle weight.

In one experiment, a tetanic contraction was obtained before and after fatiguing contractions at 10/sec. Figure 11 illustrates the lack of change seen for this contraction.

TABLE III

PHOSPHORYLCREATINE ANALYSIS DURING CONTRACTIONS AND FOLLOWING THE RECOVERY PERIOD

Animal #	1	2	3	4	5	6	7	8	9	$\bar{x}$
L: R ratio:										
a) during contractions						0.36		0.63	0.40	0.46
b) following recovery	0.83	0.96	1.0	1.11	1.08	1.02	1.26	1.09	0.83	1.02

Phosphorylcreatine is presented above as a ratio:

PC · Creatine^{-1} (left): PC · Creatine^{-1} (right)

(See text for discussion of creatine as an index of muscle weight.)

FIGURE 10. Peak rate of force development and peak rate of relaxation. See Figure 5 for significance of asterisks and numbers. Note similarity between dP/dt versus time and ΔT versus time (Figure 5).

FIGURE 10

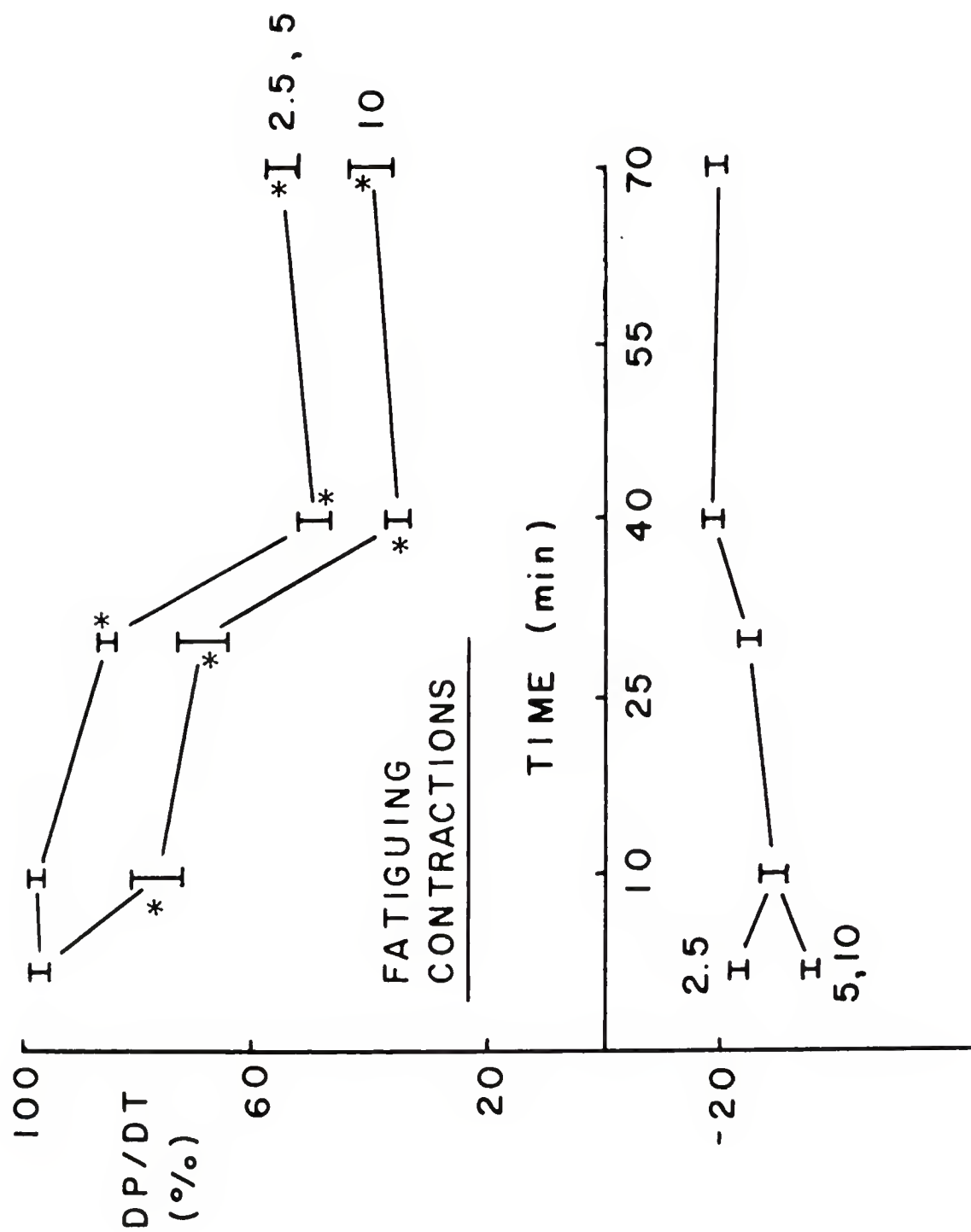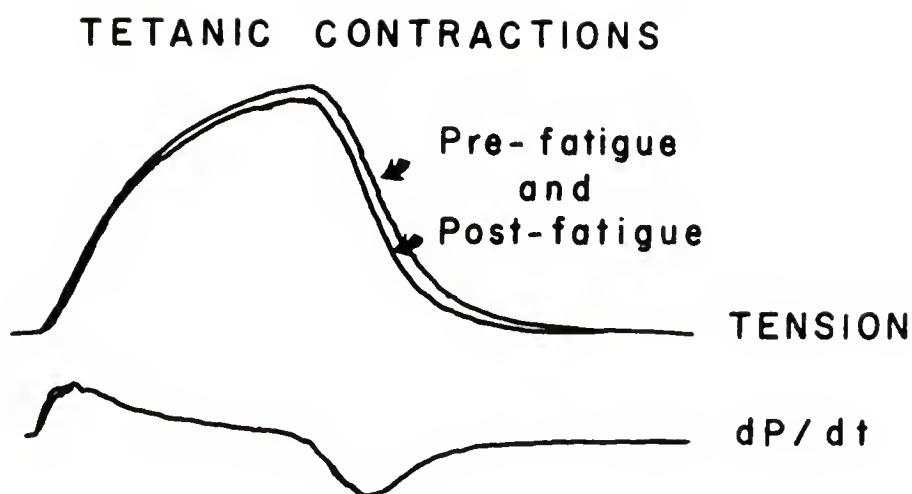FIGURE 11.   Tracings of recordings of tension and
            differential of tension for tetanic
            contractions (100/sec for 200 msec).
            Developed tension following 30 minutes
            of fatiguing contractions (10/sec) was
            only slightly lower than that before
            the 10/sec contractions. The differential
            tracer were virtually superimposable.

The tetanic contraction is recovered at a time when
twitch ΔT is still reduced.

Contractions observed during ischemia demonstrated
a reduced ΔT and a prolonged Rt 1/2. Following restoration
of blood flow, recovery of both ΔT and Rt 1/2 was 50%
complete in 30 minutes. Figure 12 shows recordings from
one muscle for contractions pre-ischemia, during ischemia
and post-ischemia. These recordings are typical for
what was seen.

Comparing the effects of dantrolene sodium, ischemia
and reduced stimulation voltage on Rt 1/2 vs ΔT (see
Figure 13), indicates that ischemia and reduced voltage
cause large alterations in Rt 1/2 with concomitant
reductions in ΔT. With administration of dantrolene
sodium, the attenuation of ΔT is not accompanied by a
substantial change in Rt 1/2. This pattern seen with
dantrolene is similar to the changes seen during the
fatiguing contractions.

Discussion

In these experiments, twitch fatigue has resulted
from 30 minutes of contractions at 2.5, 5 or 10/sec.
Forty minutes after the fatiguing contractions were
ended, no significant recovery had occurred. Recovery
would eventually have occurred following several hours
of relative inactivity (66). Twitch fatigue, then,
results from a relatively persistent alteration in the muscle
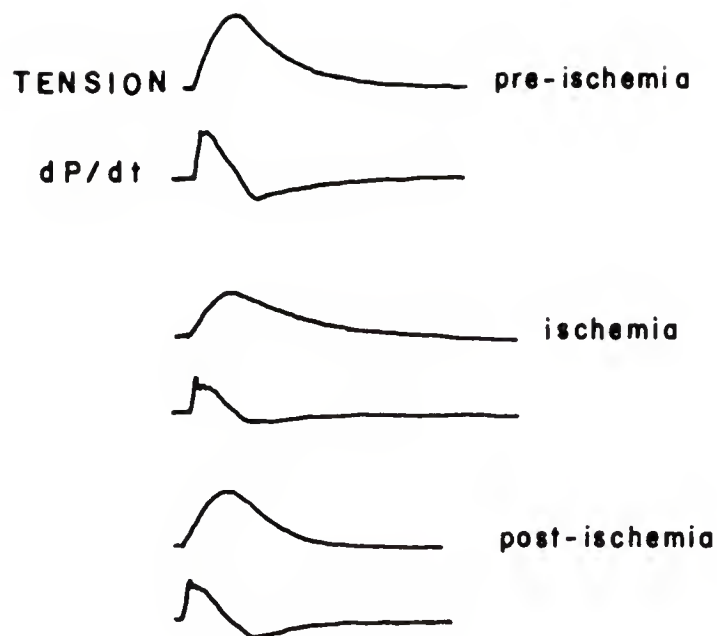which affects the contractile response to a single impulse.

FIGURE 12. Tension and dP/dt (upper and lower curves
of each pair respectively) are shown.
1) a control contraction before occlusion
   of blood flow
2) contraction during ischemia, 5 minutes
   after occlusion of blood flow
3) a post-ischemia contraction, 50 minutes
   after release of occlusion, contraction
   frequency 1/sec.
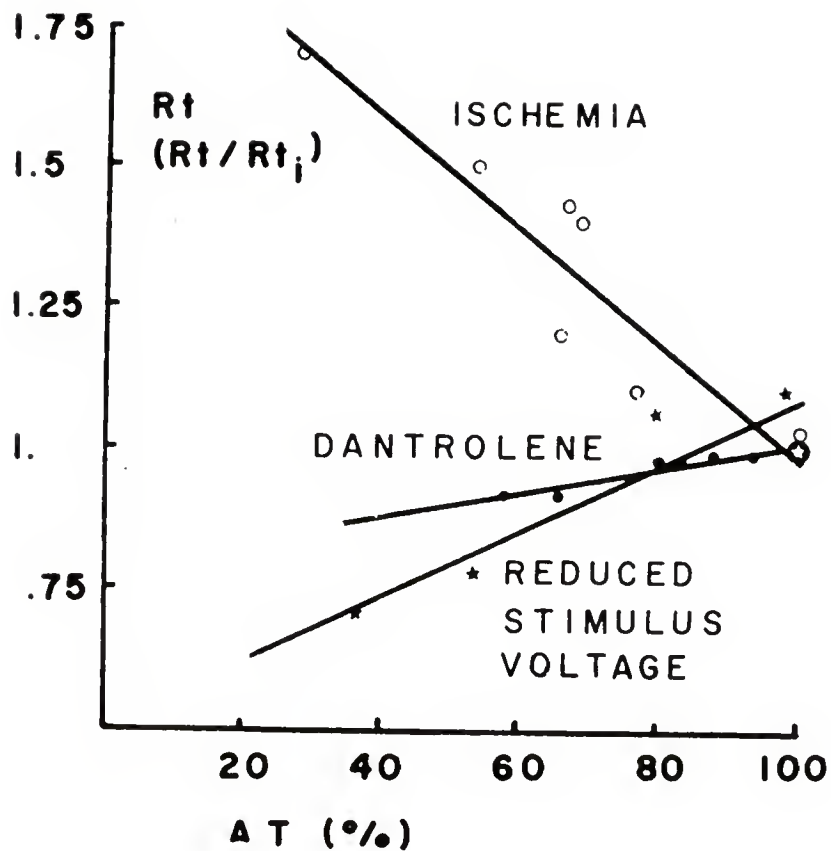
FIGURE 13. Half relaxation time versus ΛT is presented
            to illustrate the relative changes in Rt 1/2
            when ΛT is reduced by ischemia, dantrolene
            or reduced stimulation voltage. Each line
            represents one dog. Lines were determined
            by the least squares method common to all
            three lines.

The response to tetanic stimulation is not altered. Analysis of the fatigue patterns for this muscle group may provide some insight into the mechanisms responsible for this long-lasting fatigue.

The extent of comparisons between frequencies for the fatigue patterns observed in these experiments is limited. In the experiments where fatigue was caused by contractions at 5/sec or 10/sec, the frequency of stimulation was reduced to 1/sec to obtain fast traces. This procedure was implemented because full relaxation does not occur between contractions at 10/sec and 5/sec. The measurements which have been made on these contractions are affected by the resting tension. It was hoped that by reducing the frequency to 1/sec for these contractions, a true representation of the characteristics of a twitch could be obtained. Although a common frequency is used, it is evident that the preceding contractions did have an effect on the measurements (see Figure 14). The measurements made for the 2.5/sec series were made on contractions at 2.5/sec during the 30 minute fatigue period and at 0.2/sec during recovery. Due to the effect of frequency and preceding contractions on the time course of a twitch, caution must be exercised when comparing values between frequencies. Comparing the trend within one frequency with the trend within another frequency is valid.

TENSION

DIFFERENTIAL

1/sec after 10/sec
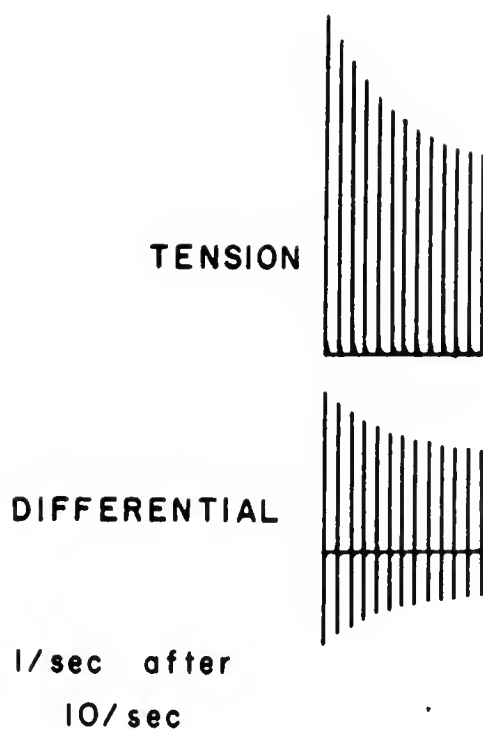
FIGURE 14.　Following fatiguing contractions at 10/sec, switching the stimulator to 1/sec results in a negative staircase. The top tracing is tension, and the lower one is dP/dt. This demonstrates the inotropic effects of previous contractions, and illustrates the mechanisms responsible for the decrease in developed tension seen following the 30 minute period of contractions. The single contraction on the left immediately follows 10/sec contractions; the others (at a reduced paper speed) were continued at 1/sec.

If contractions of the fatigued muscle are compared with contractions before the muscle was fatigued, the following characteristics are noted. Developed tension is reduced. This reduction appears to be greater when a higher frequency of stimulation occurred during the fatigue period. Contraction time and Rt 1/2 are not different in the fatigued muscle in comparison with the rested muscle for muscles fatigued at 10/sec and 5/sec. The prolongation of contraction time in recovery from fatiguing contractions at 2.5/sec is not due to fatigue, but due to the stimulation frequency during recovery. Contraction time gets shorter as frequency of stimulation is increased. For the same reason, Ct is shorter during the fatiguing contractions at 10/sec. This is an effect of previous contractions (10/sec) altering the time-course of contractions at 1/sec. A decrease was seen for dP/dt which was proportional to the decrease in ∆T. Brust (10) reported similar fatigue patterns for the mouse soleus muscle in vitro. The same author (9) reported different fatigue patterns for frog semitend-inosis muscles. The major difference was that Rt 1/2 was prolonged in the fatigued frog muscle but not in the mammalian muscles. This difference is apparently not species specific. Edwards et al. (22) reported that there is a slowing of relaxation in mouse muscle following a fatiguing effort. This slowing of relaxation was correlated with low levels of ATP (22). This can

explain the increase in Rt 1/2 seen in ischemic fatigue
(see Figure 13). Since Rt 1/2 was not prolonged following
a period of fatiguing contractions with an intact blood
supply, it would appear that ATP was available within the
muscle.

The evidence presented above suggests that the fatigue
observed in these experiments did not occur as a result
of reduced ATP levels. Other evidence supports this
suggestion. Although PC levels were low during the
contraction period, (see Table III), resynthesis had
occurred before significant recovery of ΔT. Rapid
resynthesis of PC during recovery from a period of
contractions was also observed by Piiper and Spiller (51).
There seems to be no direct relationship between PC levels
and ΔT. This is contrary to a report by Spande and
Schottelius (56), but supports the observations of Fitts
and Holloszy (29). It should also be pointed out that
glycogen was still available after 30 minutes of stimulation
at 10/sec (14) and lactate production had declined (or
even reversed - lactate uptake) (60). It would seem that
energy was available, but demand for energy was reduced.
In support of this conclusion, PvO_2 was not below minimum
critical PO_2 (59) at any time that PvO_2 was measured.

The muscle is capable of developing more force than
that seen in a twitch. Twin impulse stimulation causes
developed tension to be double that seen with single

impulse stimulation (34). This relationship is maintained for rested as well as fatigued muscles. It would appear that the reduced ΔT of the fatigued muscle was due to reduced activation.

A reduced activation of skeletal muscle could, theoretically be the result of: a) reduced neuromuscular transmission, b) reduced Ca^{2+} release, or c) reduced responsiveness of the contractile elements to Ca^{2+}. Neuromuscular transmission appears to be intact (34). This agrees with others (3, 35, 37) who have found only minimal changes in muscle action potentials or electro-myographic response following comparable stimulation periods. There is a possibility that Ca^{2+} release has been attenuated. This could cause a reduced ΔT and dP/dt without altering Ct or Rt 1/2 (15). Brust (10), who observed similar fatigue patterns with the mouse soleus muscle, suggests that the fatigue he observed was a result of reduced Ca^{2+} release (see also (63) for the converse). The factor(s) responsible for the reduced Ca^{2+} release is/are not known. Bonner et al. (5) have reported that muscle mitochondria accumulate Ca^{2+} during exercise. This would reduce the pool of Ca^{2+} available for recycling in the sarcoplasmic reticulum (67) and consequently limit the amount available for release.

Dantrolene sodium apparently reduces the amount of Ca^{2+} released per impulse (24). This drug was used in these experiments to determine the effects of reduced

Ca^{2+} release on a twitch. A dantrolene treated muscle contracts with a time-course similar to the fatigued muscle. Ischemia or reduced stimulation voltage caused a reduction in ΔT, but this was accompanied by changes in Rt 1/2.

An alternative hypothesis involves a reduced binding of Ca^{2+} to the regulatory proteins. During contractions, muscle pH probably falls (33). Fuchs et al. (32) has shown that binding of Ca^{2+} to troponin is inhibited by lower pH. Fitts and Holloszy (30) has suggested this may be a mechanism responsible for the fatigue seen in their experiments.

The experiments reported herein do not permit discrimination between these two potential mechanisms of fatigue. The fatigue observed following 30 minutes of stimulation at 2.5, 5 or 10/sec appears to be a result of reduced activation. Further experiments will need to be conducted to determine which of the theories described above applied to these muscles.

EFFECTS OF RESPIRATORY ACIDOSIS ON THE TWITCH CONTRACTION

Introduction

Twitch developed tension is attenuated following a period of repetitive stimulation. This attenuation is apparently the result of a reduced activation, due either to a reduced release of Ca^{2+} from the lateral sacs or from a reduced responsiveness of the contractile proteins. It is not due to a reduced availability of energy (ATP, PC). Fitts and Holloszy (30) have suggested that the reduced twitch response is a result of inhibition of the contractile process due to a reduced intracellular pH. Steinhagen et al. reported evidence supporting this hypothesis (61). He reported that dog gastrocnemius muscle fatigues more quickly during respiratory acidosis than during normal pH balance.

Specific mechanisms which may contribute to this acidosis-induced fatigue have been proposed. Nakamura and Schwartz (46) reported that uptake of Ca^{2+} by sarcoplasmic reticulum is accelerated in low pH medium. This could reduce the duration of activation for a twitch by reducing the Ca^{2+} concentration more quickly. Fuchs et al. (32) have reported that Ca^{2+} binding to troponin is inhibited by H^+. These molecular mechanisms, if effective under physiological conditions would cause a reduction in ΔT, fatigue.

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The purpose of this study was to observe the effects of acidosis on the developed tension and the time-course of a twitch contraction of the in situ dog gastrocnemius-plantaris muscle. Intracellular pH can be reduced more easily via respiratory acidosis than by metabolic acidosis (12). For this reason, acidosis was induced by reducing the ventilatory rate. The results of this study indicate that acidosis is unlikely a direct cause of twitch fatigue.

Methods

The gastrocnemius-plantaris muscle preparation as described in the General Methods section was used in this series of experiments.

In each experiment, the nerve was stimulated at a frequency of 0.2/sec. In three experiments, ventilation was controlled to maintain arterial pH near 7.4 for the duration of the experiment (2 hours). In four experiments, after the muscle had been contracting (0.2/sec) for 20 minutes, ventilation was reduced to 3-4 breaths/minute. The mixture of inspired gas was adjusted (with 95% O_2 and 5% CO_2) to maintain normal arterial PO_2 during the hypoventilation. The period of hypoventilation was continued until arterial pH was less than 7.1 (60-90 minutes). This period of hypoventilation was followed by a period of hyperventilation (20 breaths/minute). The hyperventilation was continued for 40-60 minutes (see Figure 15).

With an additional four dogs, the sequence of ventilatory adjustment was reversed (control, hyperventilation, hypoventilation) to permit evaluation of a possible order effect.

At ten minute intervals throughout each experiment, arterial and venous blood samples were obtained (0.8 ml) in glass tuberculin syringes (1 ml capacity). The samples were sealed with mercury containing caps and kept in ice until they were analyzed for PO_2, PCO_2 and pH (Radiometer, Copenhagen).

Fast traces of contractions were also obtained at 10 minute intervals (200 mm/sec on Gould-Brush Model 2400 recorder). The fast traces were measured for developed tension (ΔT), half relaxation time (Rt 1/2), contraction time (Ct), peak rate of force development (dP/dt) and peak rate of relaxation (-dP/dt) (see Figure 6).

Statistical analysis was by a two way analysis of variance for repeated measures (2). For statistical analysis, the last two measurements (fast traces or blood gases) before alteration of the ventilation were utilized to represent the state in which they occurred (see Figure 15).

Results

After 20 minutes of contractions, ventilation was reduced to 3-4 breaths per minute (Group A) or increased to 20 breaths per minute (Group B). This adjustment in ventilation resulted in a reduction in arterial pH from

FIGURE 15.   Developed tension, PO_2, ventilation and
             arterial pH for one dog, from Group A.
             Blood samples and fast traces obtained
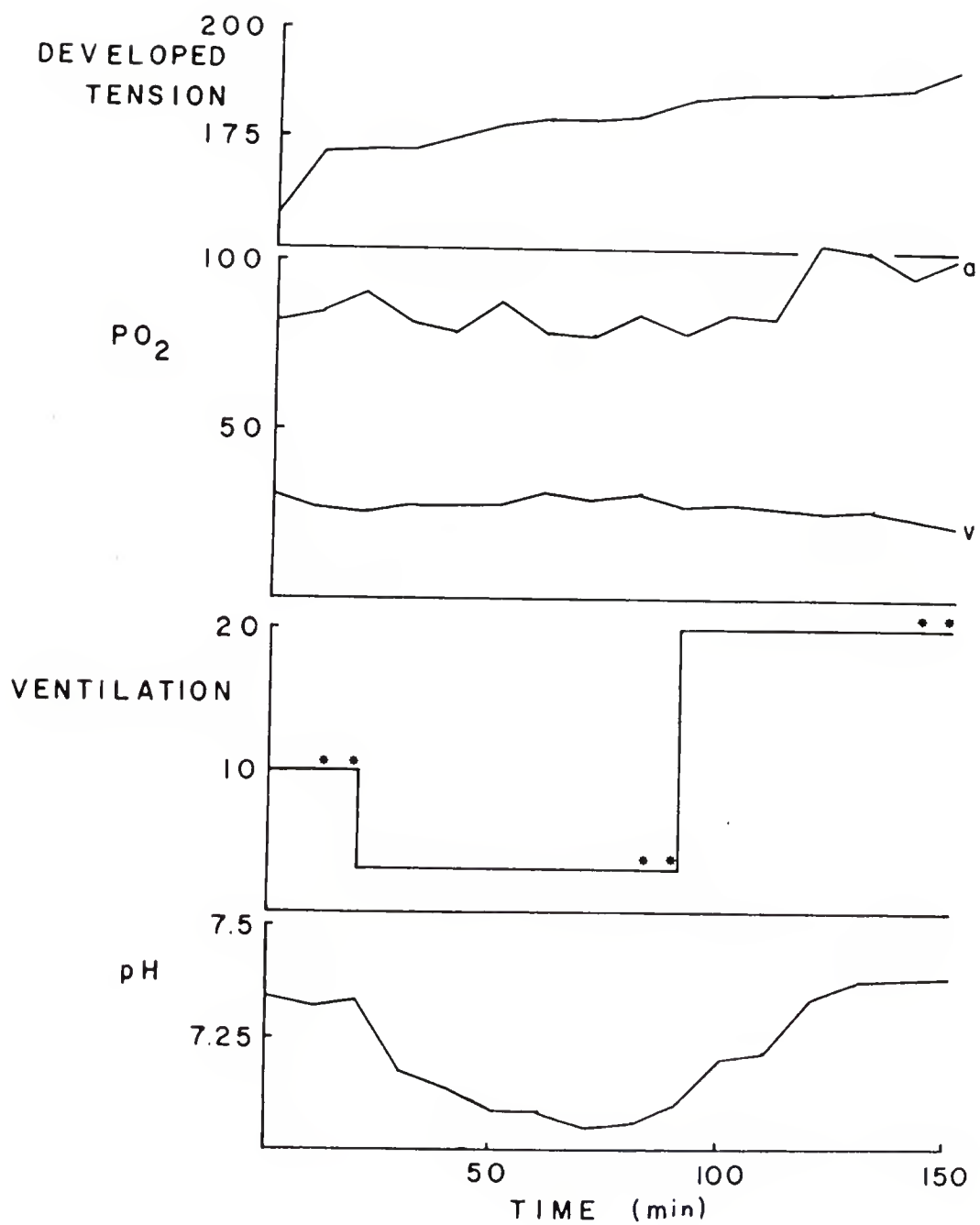   at * were used for statistical analysis.

FIGURE 15

7.37 $\pm$ .01 (mean $\pm$ SEM) during the control period to

7.08 $\pm$ .03 in Group A and an increase in Group B from

7.36 $\pm$ .02 to 7.52 $\pm$ .02. After 60-90 minutes, ventilation

was increased to 20 breaths per minute in Group A and

reduced to 3-4 breaths per minute in Group B. This

second alteration in ventilatory frequency resulted in

an increase in pH to 7.4 in Group A and a reduction in

Group B to 7.2 (see Figure 16). In Group C, ventilation

was not altered, and arterial pH did not vary during the

experiments. The reduction in ventilation did not

significantly decrease arterial PO_2.

When arterial pH remained constant for 2 hours

(Group C), ΔT increased with time. By the end of 2 hours,

ΔT was greater than it had been at the first 20 minute

period. As illustrated in Figure 17, ΔT increased with

time in Group A also but not in Group B. The only

significant difference in Groups A and B was that ΔT

during hyperventilation was greater than ΔT at any other

time period in Group A and greater than control in Group

B (see Table IV).

Despite the apparent effect of hyperventilation on ΔT,

there was no significant correlation of ΔT with arterial

or venous H^+ concentration (see Table V). Furthermore,

ΔT was not reduced during hypoventilation. When arterial

pH fell to 7.09 $\pm$ .03, ΔT did not change significantly.

In Group C, dP/dt did not change significantly. In

Group A and Group B, however, dP/dt was higher during

TABLE IV

STATISTICS FOR DIFFERENCES BETWEEN VENTILATION STATES FOR EACH GROUP

	Group A		Group B		Group C	
		Rank		Rank		Rank
ΔT	.006	3 $\overline{2\ 1}$	.05	$\overline{3\ 2}$ 1	.04	3 $\overline{2\ 1}$
Rt 1/2	.18	NS	.003	$\overline{1\ 2}$ 3	.99	NS
Ct	.86	NS	.005	$\overline{1\ 3}$ 2	.98	NS
dP/dt	.001	3 2 1	.001	$\overline{3\ 2}$ 1	.122	NS
-dP/dt	.002	3 $\overline{1\ 2}$	.02	$\overline{3\ 2\ 1}$	.91	NS

The p values are given for ΔT, Rt 1/2, Ct, dP/dt and
-dP/dt for Group A (control, hypoventilation, hyper-
ventilation), Group B (control, hyperventilation,
hypoventilation) and Group C (control). Means are
listed under the rank order. Rank indicates the
order from largest to smallest for Control, 1,
Acidosis, 2, and Alkalosis, 3. For Group C, rank
1, 2 and 3 indicate time periods; first 20 minutes,
1, next 60 minutes, 2, and last 40 minutes, 3.
Horizontal bars across ranks indicate means which
are not significantly different. NS indicates that
means are not significantly different.

FIGURE 16. Arterial and venous H^+ concentration during ventilatory states. The three columns on the left are from Group A. The three columns on the right are from Group B. Open columns are arterial $[H^+]$. Vertical bars are one standard error of the mean. Upper bar is for venous, lower bar for arterial SEM.
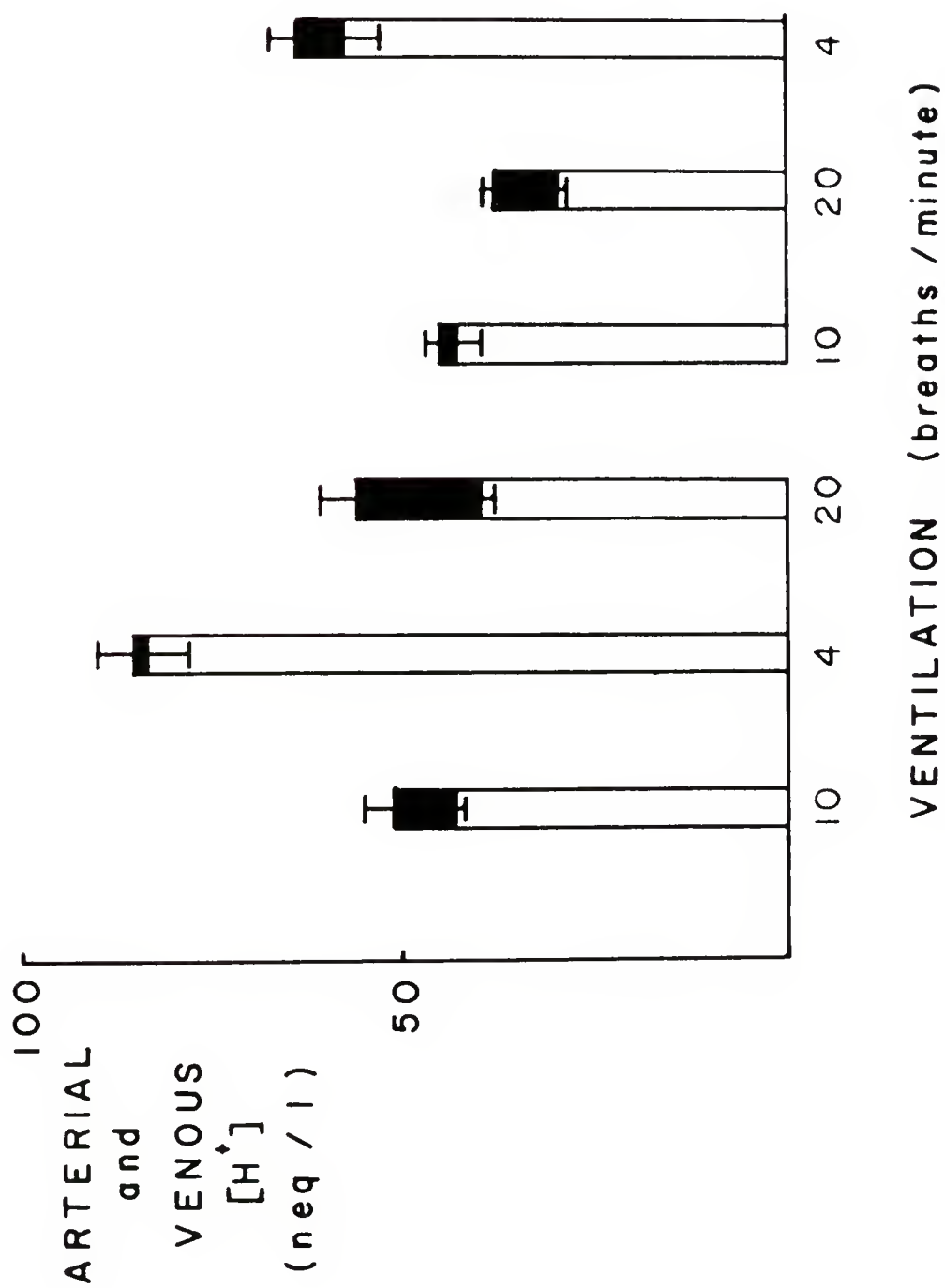
FIGURE 16

FIGURE 17. Mean arterial and venous PO_2 during the different ventilatory states. The three columns on the left represent Group A. The three on the right represent Group B. Open bars represent venous PO_2 and combined (open and closed) bars represent arterial PO_2. Vertical bars represent one SEM.
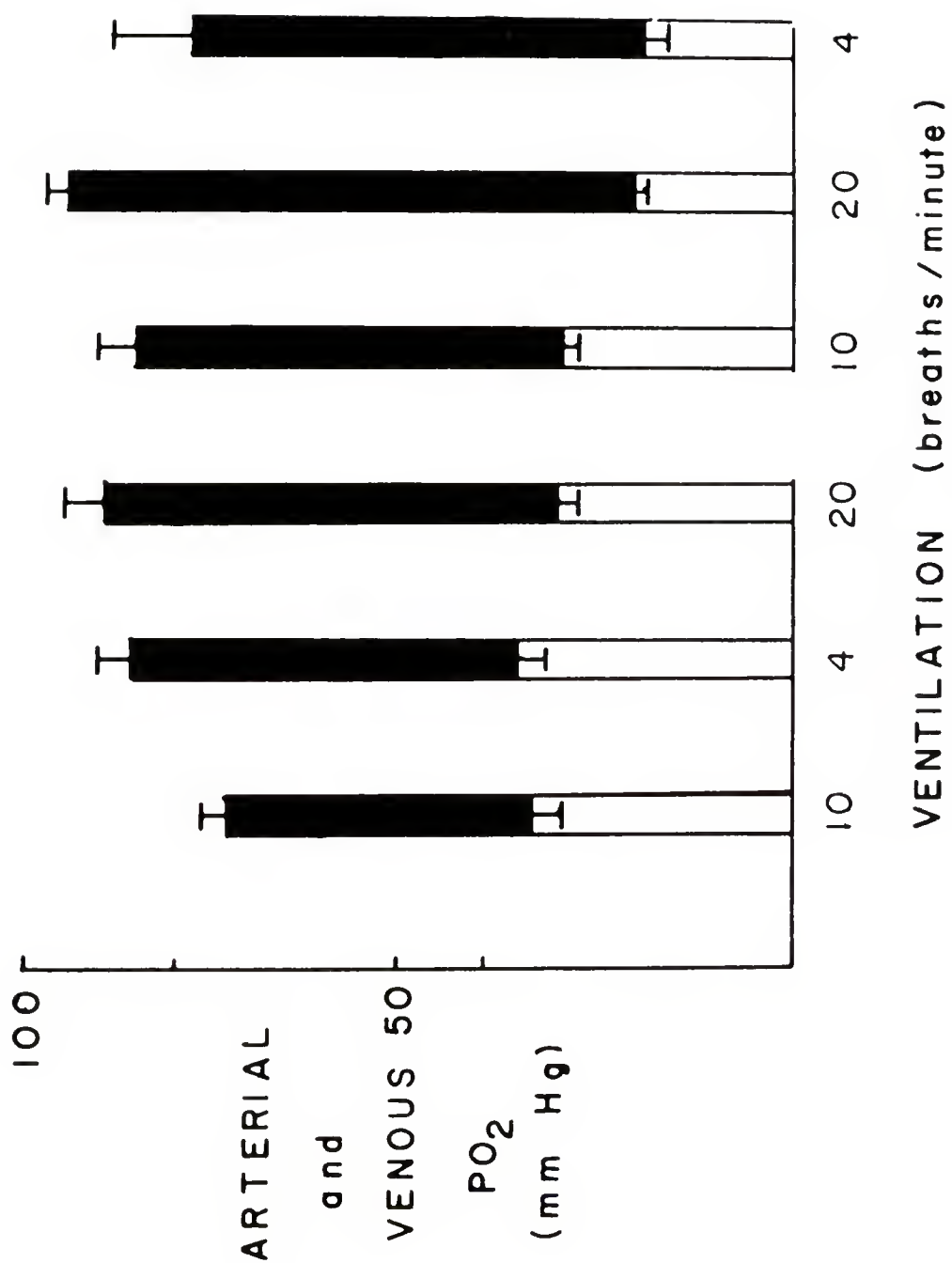
FIGURE 17

hyperventilation than during the initial control period
(see Table I). There was no significant correlation
between arterial pH and dP/dt. This suggests, as seen
for ΔT, that the alterations in dP/dt associated with
ventilation patterns are not directly related to pH
(see Figure 18).

The peak rate of relaxation was greatest during
hyperventilation. In Group A, this was significantly
greater than both the control period and the period of
hypoventilation. In Group B, however, where arterial
pH increased to 7.52 during hyperventilation (as opposed
to 7.4 in Group A), -dP/dt during hyperventilation was
greater than that during normal ventilation, but not
significantly different from that during hypoventilation.
There was no significant correlation between -dP/dt and
arterial $[H^+]$.

In Groups A and C there were no significant changes
in Ct or Rt 1/2. In Group B, however, Ct was shorter
during hypoventilation than at other times. Rt 1/2 was
shorter during hyperventilation than at other times in
Group B (see Figure 19).

Discussion

It has been suggested that intracellular acidification
is the cause of skeletal muscle fatigue (30). If there
is a direct influence of pH on the force of contraction,
this phenomenon would be independent of the manner in
which the pH change was obtained. It is apparent in

FIGURE 18.   Mean developed tension, dP/dt and -dP/dt
             during different ventilatory states.
             Developed tension is % of highest in each
             experiment. dP/dt and -dP/dt are % of
             highest dP/dt in each experiment. Vertical
             bars represent one standard error of the
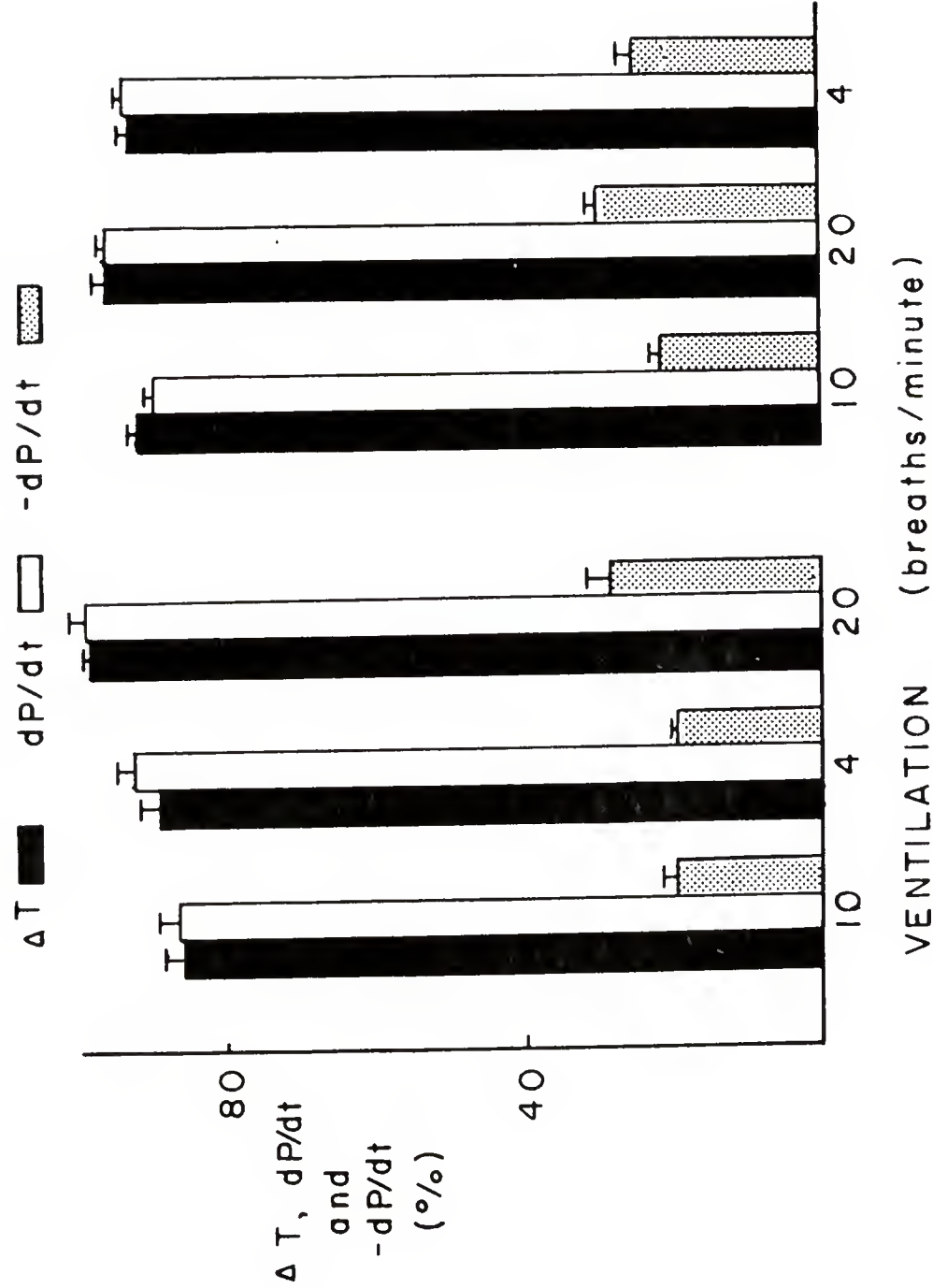   mean.

FIGURE 18

FIGURE 19. Mean contraction time and Half relaxation time during different ventilatory states. Vertical bars represent one SEM.
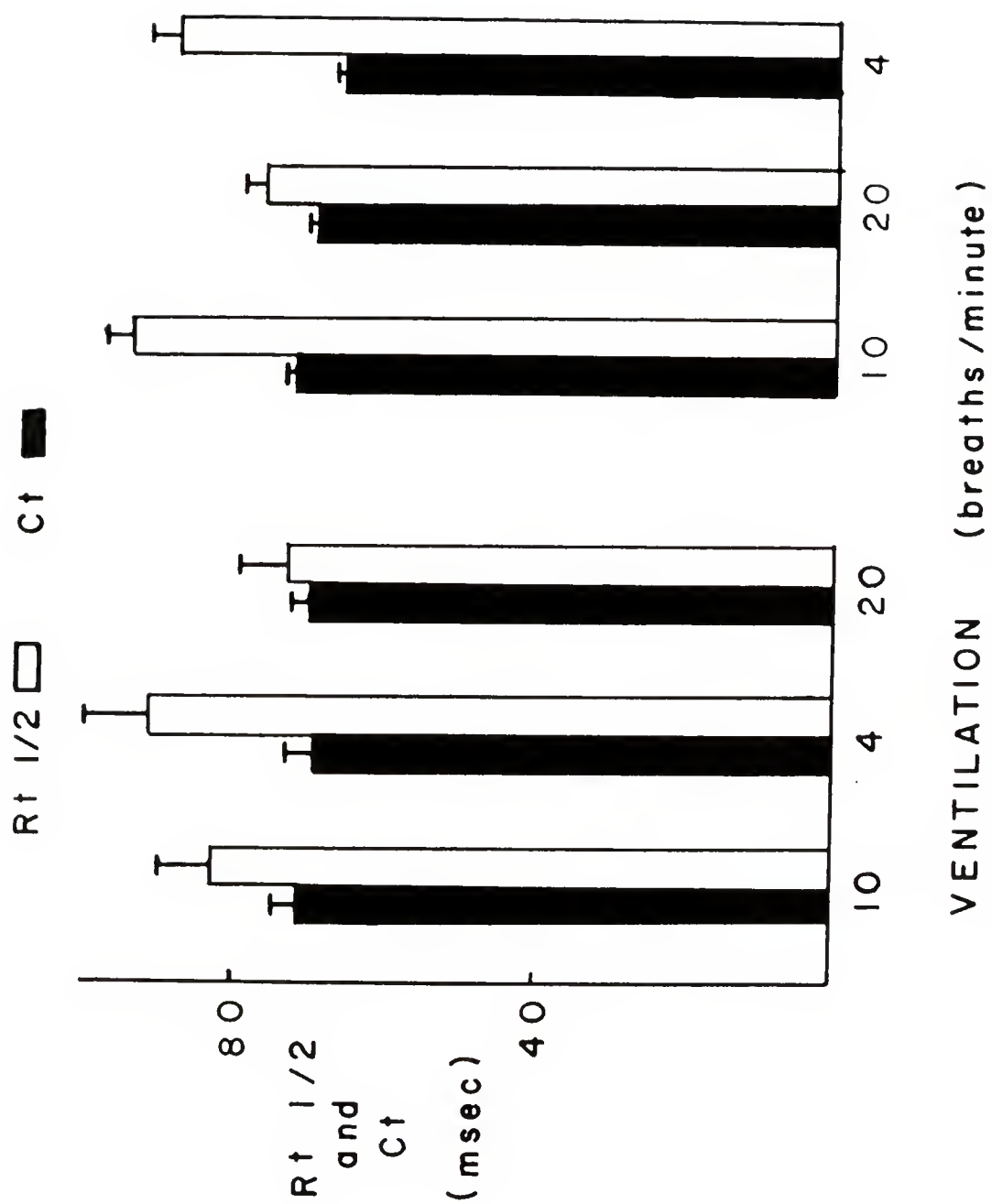
FIGURE 19

TABLE V

STATISTICS FOR TWITCH CHARACTERISTICS VERSUS BLOOD GASES

	Group	T p=	R^2=	Rt 1/2 p=	R^2=	dP/dt p=	R^2=	-dP/dt p=	R^2=	Ct p=	R^2=
Art [H^+]	A	.4		.001	.88	.8		.09		.17	
	B	.25		.06		.26		.07		.1	
	C	.33		.29		.4		.15		.22	
ven [H^+]	A	.78		.002	.85	.72		.2		.31	
	B	.77		.13		.75		.23		.03	.56
	C	.59		.26		.11		.24		.26	
$PaCO_2$	A	.25		.001	.89	.57		.05	.52	.13	
	B	.04	.56	.04	.44	.04	.4	.01	.75	.25	
	C	.2		.13		.15		.04	.89	.06	

Significance values are given for correlations between twitch characteristics and blood gases. R^2 values indicating the percent of variability explained by the variable are given for significant correlations (p<.05). Groups are as defined in the legend for Table IV.

these experiments that the changes in Ct, Rt 1/2 and
-dP/dt are not directly associated with arterial pH.
This complication will be discussed further below.
However, to give consideration to the possibility that
acidosis causes fatigue, the results from Group A will
be discussed with respect to the likelihood that the
hypoventilation in these experiments resulted in alterations
in intracellular pH comparable to what might be expected
from contractions at 10/sec for 30 minutes.

In these experiments, arterial pH was reduced from
7.37 to 7.08 by hypoventilation. The acidosis accompanying
the hypercapnia was not associated with any change in ΔT,
Rt 1/2 or Ct. It would appear that the fatigue described
earlier cannot be a result of acidosis, unless intra-
cellular pH during the fatiguing contractions changes
more than it did during hypoventilation.

The magnitude of the intracellular pH change occurring
in these experiments can be estimated from the results of
Burnell (12). Hypercapnia in dogs ($PaCO_2 = 55$ mm Hg)
resulted in a reduction of intracellular pH of neck
muscles from 6.85 to 6.57. Intracellular pH was
determined by the DMO method (12). Burnell (12)
observed that the maximal response had occurred within
15 minutes. In the experiments reported herein, arterial
PCO_2 increased to 57 ± 3.7 mm Hg. The measurements
presented were taken 60-90 minutes after the alteration
in ventilation. It is reasonable to assume that a very

similar change in intracellular pH occurred in these experiments as was observed by Burnell, under almost identical circumstances. It can therefore be concluded that a change in intracellular pH values from normal resting values (approximatley 6.85) to 6.57 does not result in a change in developed tension.

The important point, however, is whether or not intracellular pH fell to this level or lower during the fatiguing contractions reported in earlier chapters. Estimates of intracellular pH changes under these circumstances can only be tentative. Venous PCO_2 during the fatiguing contractions was never greater than 57 mm Hg (mean = 49 mm Hg at t = 10 minutes for 10/sec). This hypercapnia would not cause an acidosis sufficient to reduce ΔT (if reduced pH will cause reduced ΔT!). However, there is lactic acid production during the first 5-20 minutes of this type of stimulation (60). This is likely to contribute to an intracellular acidification. Sahlin et al. (53) report that in humans, performing maximal exercise to exhaustion, intracellular pH drops from 7.08 to 6.6. This is comparable to that seen by Hermanson and Osnes (39). This decrease in pH was associated with an increase in lactic acid production. Following the bout of exercise, intracellular pH recovered to 7.0 within twenty minutes. It is unlikely that intracellular pH changed as much in the 10/sec fatigue as it did during the exhausting exercise reported by Sahlin et al. (53). Furthermore, it seems

likely that intracellular pH would have returned to resting levels after 40 minutes of recovery. Developed tension at this time is still very much reduced (i.e., no significant recovery has occurred). It seems reasonable to conclude that the persistent fatigue caused by contractions at 10/sec for 30 minutes does not result directly from acidosis. There may, however, be indirect ways in which intracellular pH may affect the contractile process, resulting in a change within the muscle which persists beyond the time when pH has returned to control (26).

It is evident that changes in ventilatory pattern do affect muscle contraction. During hyperventilation, ΔT was increased. This was the case whether hypoventilation preceded the period of hyperventilation or if normal ventilation preceded it. In the former case (hypoventilation preceding) arterial pH returned to 7.4, so there was no absolute arterial alkalinization. The increase in ΔT under these circumstances was greater than the increase seen in the latter case (hyperventilation preceded by normal ventilation), despite the fact that this procedure resulted in an increase in arterial pH to 7.52. It seems that hyperventilation increases ΔT, but hypoventilation only reduces ΔT when it is preceded by hyperventilation.

The high p values reported in Table V reflect the lack of relationship between ΔT and $[H^+]$. The fact that significant differences were observed between ventilation

periods is due not to absolute pH changes, but relative changes possibly in conjunction with some other change (ion distribution? i.e., see 26, 31) associated with changes in ventilation.

The changes seen in Ct, dP/dt, -dP/dt and Rt 1/2 also suggest that alterations in ventilatory pattern can affect contraction. The mechanisms responsible for these changes are not clear.

In this study, it is likely that hypercapnia resulted in intracellular acidosis. This acidification was not accompanied by a reduction in ΔT. It can be concluded that fatigue is not caused by acidosis if fatiguing contractions do not cause any greater acidification than that which occurred in these experiements.

SUMMARY

O$_2$ Uptake and Developed Tension

The amount of oxygen used by a muscle was proportional to the amount of tension developed. This occurred over a wide range of forces when ΔT was altered by any of the following:

a) Fatigue, after 30 minutes of stimulation at 14-20/sec

b) Fatigue, during fatiguing contractions at 3-6/sec

c) Twin impulse stimulation, before and after fatigue at 14-20/sec

d) Fatigue, during contractions at 1/sec without blood flow (ischemic fatigue)

e) Attenuated contraction, caused by administration of curare in sufficient doses to reduce the force of contraction by as much as 70%.

These results suggest that the major determinant of energy utilization during an isometric contraction is the magnitude of the developed tension. It should be emphasized that these were twitches or very brief tetanic contractions in which developed tension rose then fell, but did not maintain a plateau of tension and can therefore be considered to have a minimal "tension maintenance" component to the determinants of energy utilization. The possibility that neuromuscular junction failure may have contributed to the observed fatigue was tested. It was demonstrated that transmitter release was normal after 30 minutes of stimulation at 20/sec.

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Time-course of the Twitch Contraction in Fatigue

The time-course (Ct and Rt 1/2) and the rate of change of force (dP/dt and -dP/dt) were observed when muscles were fatigued with contractions at 2.5, 5 and 10/sec for 30 minutes. The pertinent observations are listed below.

a) Developed tension fell more rapidly during 10/sec stimulation than 2.5 or 5/sec.

b) In the 40 minutes following the fatiguing contractions, no significant recovery of ΔT occurred.

c) The Ct and Rt 1/2 of a fatigued muscle are no different from those of a rested muscle.

d) The dP/dt is greatly reduced in fatigue and is significantly correlated with ΔT. A reduction in -dP/dt is also seen in the fatigued muscle.

e) Despite the persistence of fatigue, the following parameters have returned to pre-contraction values after 40 minutes of recovery: venous pH, PO_2, $\dot{Q}$ and muscle phosphorylcreatine concentration.

It has been concluded from the above observations that fatigue is not caused by a lack of availability of energy. Fatigue appears to be the result of a reduced activation of the muscle cells. This may be due to either a reduced amount of Ca^{2+} released or a reduced sensitivity of the contractile proteins. Since ΔT of a tetanic contraction of the fatigued muscle was not different from that of a rested muscle, it appears that the capacity of the muscle to generate force is not reduced. Fatigue, then must be a result of lower Ca^{2+} release or a change in the binding relationship between Ca^{2+} and troponin (less Ca^{2+} bound at a given Ca^{2+} concentration).

Acidosis and the Twitch Contraction

When acidosis was induced by hypoventilation, developed tension did not decrease. It is likely that the intra-cellular pH reached a level in these experiments comparable with that which was reached in the experiments summarized above. This suggests that the reduced intensity of activation observed as a result of fatiguing contractions was not a result of intracellular acidosis.

PROPOSED HYPOTHESES FOR SKELETAL MUSCLE FATIGUE

Several hypotheses can be invoked to explain how there may be less Ca^{2+} released from the lateral sacs. Following is a discussion of a few hypothetical mechanisms to explain the fatigue. These mechanisms are presented, not as an explanation for the observed fatigue, but as hypotheses which need to be tested to prove or disprove their involvement in this fatigue. A detailed hypothesis of the fatigue seen in these experiments is not justified by the data available.

Depletion of Calcium at Lateral Sacs

Less Ca^{2+} would be released from the lateral sacs if less Ca^{2+} were located at the lateral sacs. This situation would occur if Ca^{2+} were lost from the cell or sequestered by some other organelle within the cell. It is unlikely that significant Ca^{2+} was lost from the cell during this period of stimulation. The Ca^{2+} concentration of the interstitial fluid is high compared with the intracellular concentration at rest or during contractions (18).

The amount of Ca within each muscle cell may be unaltered in fatigue. Under these circumstances, the amount of Ca^{2+} available to the lateral sacs may be limited if some organelle sequesters Ca^{2+}. It has been reported that mitochondria accumulate Ca^{2+} during exercise

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(5). It is conceivable that calcium accumulation by mitochondria may reduce the amount of Ca^{2+} available for recycling in the sarcoplasmic reticulum. This could reduce the amount of Ca^{2+} released with each impulse. Other organelles may also sequester Ca^{2+}, limiting the amount of Ca^{2+} available to the sarcoplasmic reticulum.

The longitudinal tubules are known to accumulate Ca^{2+} (67). It is unlikely that this accumulation would permit the observed persistence of fatigue. Apparently the time necessary for translocation of the Ca^{2+} from the longitudinal reticulum to the lateral sacs is much shorter (67) than the observed 40 minutes of recovery during which no significant increase in ΔT occurred.

Compartmentalization Within the Lateral Sacs

A reduced Ca^{2+} release from lateral sacs would occur if Ca were compartmentalized within the lateral sacs in a manner which made it unavailable for release (i.e., bound vs free). There is no specific evidence available to support this hypothesis. It is mentioned here only because it is a hypothetical explanation of the data.

Attenuated Trigger Mechanism for Release of Calcium

A third potential mechanism which would result in reduced Ca^{2+} release would be an attenuation of the trigger mechanism for release of Ca^{2+}. This mechanism is poorly understood and therefore a discussion of how this might be affected in fatigue is unwarranted. One possibility, however, would be that conduction of the

muscle membrane depolarization into the transverse tubular system is altered in a manner which affects the trigger mechanism and therefore reduces Ca^{2+} release.

Reduced Binding Sensitivity for Calcium

It is possible that the amount of Ca^{2+} released from the lateral sacs is unaltered, but the degree of activation accomplished at that concentration is reduced. It has been observed that acidosis was not likely the cause of this alteration, but other metabolic factors may be capable of altering the binding relationship between Ca^{2+} and troponin, thereby reducing the intensity of activation at a given free Ca^{2+} concentration.

CONCLUSIONS

It is apparent that further research will be necessary to elucidate the mechanism causing the fatigue observed in the experiments reported herein. This research, however, has eliminated several proposed mechanisms of fatigue. A few hypotheses are presented which may contribute to the fatigue observed in these experiments. Each of these hypotheses is based on a central theme, a reduced activation of the contractile proteins. It remains to be seen which of these mechanisms (if any) is the actual cause of the twitch fatigue observed in these experiments.

APPENDIX

Method for Phosphorylcreatine Analysis

The method of Ennor and Stocken (25) was used to determine phosphorylcreatine content of muscle samples. This method determines creatine content before and after a period of acid hydrolysis of phosphorylcreatine. The key reaction for this determination is creatine with diacetyl which yields a pink colored compound. The intensity of the pink color is proportional to creatine concentration when diacetyl is available in excess. The optical density is determined with a spectrophotometer at 525 nm.

Chemicals and Reagents for Deproteinization and Determination

I   Perchloric Acid, stock 70-72% (Baker)
     a) .8 M in 40% ethanol
     b) .6 M (no ethanol)

II   Potassium Carbonate, (Baker) 3 M containing
     .5 M triethanolamine (Baker)

III Sodium Hydroxide (Malinkrodt)
     a) 1 N
     b) .4 N

IV   Hydrochloric acid (Fisher)
     a) 1 N
     b) .4 N

V   p Chloromercuric acid (Sigma) (also called p
     Hydroxymercuric acid) .05 M purchased as
     solution, or as powder then dissolved in
     NaOH (1 N) then made to volume with H_2O.

VI   Naphthol (Sigma) 1% in stock alkali, mix new
     daily

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VII Diacetyl (Sigma)
   a) stock solution 1%
   b) mix 1:20 daily for use in assay

VIII Stock Alkali
   for 1 liter: 160 g Na_2CO_3 (Baker) and 60 g NaOH
   (Malinkrodt)

IX Creatine (Sigma) (for standards)
   16 μ g/ml

Procedure for Deproteinization

Muscle samples weighing 30-80 mg were frozen in situ with metal clamps pre-cooled in liquid N_2. The muscle samples were kept frozen until they were homogenized in Perchloric acid. The samples were homogenized (Vertis) for 60 seconds at high speed in 6 ml of ice cold .8 M Perchloric acid. The homogenate was poured into a centrifuge tube (15 ml) and the blades and bown were rinsed with 3 ml of .6 M Perchloric acid. The .6 M Perchloric acid was retained in a separate centrifuge tube.

The homogenate was spun down at 1200 g for 15 minutes at 0°C. The supernatant was saved, and the pellet was resuspended in the 3 ml rinse (.6 M Perchloric acid). This suspension was recentrifuged at 1200 g for 15 minutes and the supernatants were combined.

The combined supernatant was neutralized to pH 5.5-6.0 with addition of 3 M Na_2CO_3. This was added dropwise with constant mixing to avoid bubbling over. Samples were subsequently certrifuged at room temperature in a desk-top centrifuge at maximal rpm for 10 minutes. The volume of the supernatant was determined and the

supernatant was stored in a freezer until further analysis
was done (usually same day, but a delay of 2-3 days made
no difference).

Determination of Free and Total Creatine

Duplicate analysis for free and total creatine were
made on each sample (4 aliquots per supernatant). Standards
also in duplicate were run with each determination.
Following, is a list of the procedures for free and total
determination:

1. Neutralized samples to pH = 7.0-7.3 with 1N
   NaOH (and 1N HCl if needed).

2. Measure 4 aliquots (.5-1 ml) of each supernatant
   into graduated tubes.

3. Add H_2O to 3 ml mark.

4. Place 2 (of 4) tubes in hot water bath (65°C)
   to equilibrate.

5. Add 1 ml of .4 N HCl to tubes which have
   equilibrated to 65°C.

6. Replace in hot water bath for 9 minutes. These
   are tubes for total creatine determination.

7. Remove the tubes from the bath and add 1 ml of
   .4 N NaOH. Place in ice bath to cool quickly
   to room temperature.

8. To each of the four tubes (2 free and 2 total),
   add sequentially: 1 ml of p hydroxymercuribenzoate,
   2 ml of Naphthol and 1 ml diacetyl.

9. Make volume to 10 ml, agitate and place in dark
   for 20 minutes.

10. Read optical density at 525 nm.

Standards receive the same treatment as the samples
for free creatine (blank does not get 1 ml p hydroxy-
mercuribenzoate. Creatine content of samples is

determined from the regression line obtained from the standards. Phosphorylcreatine content is (total creatine - free creatine)/total creatine.

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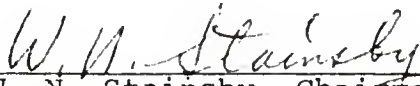   J. Gen. Physiol. 51: 65-83, 1968.

BIOGRAPHICAL SKETCH

Brian Robert MacIntosh was born in Owen Sound, Ontario, March 26, 1952. He graduated from the Owen Sound Collegiate and Vocational Institute in 1971. He received his Bachelor of Science degree in Human Kinetics in 1975 at the University of Guelph.
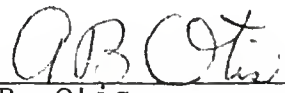
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Brian is married to the former Patricia Dale Wilkie. They have two children. Jennifer Louise and Robert John David.
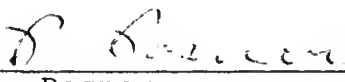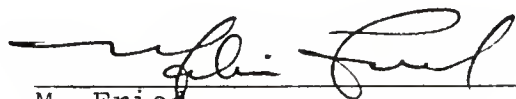
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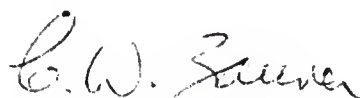W. N. Stainsby, Chairman
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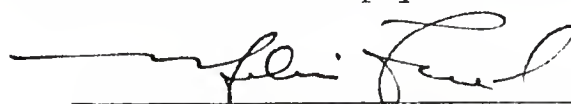
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June, 1979

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